

Put the horse before the cart

This week's European summit meeting at Milan is an opportunity both for stimulating technical collaboration and to create a muddle. Governments should temper enthusiasm with cold calculation.

EUREKA, President François Mitterrand's way of saying that he has seen the light for Europe, will occupy the European heads of government at their meeting in Milan this weekend. The plan is to invest common resources in technological developments that will help to bridge what Europeans used, in the 1960s, to call the "technology gap" between themselves and the United States. (Nowadays, Japan is as much in people's minds.) President Mitterrand has been preaching strength through technological development ever since he took office four years ago, but his most recent advocacy of this cause appears to have been stimulated by the fear that the US Strategic Defense Initiative, whatever its military implications, cannot fail to widen the gap between the competence of European companies and those in the United States. The discomfort of many European governments with the military and political implications of the US proposals has given Eureka a more hearty welcome than the earlier French initiatives (see *Nature* 6 June, p.443). It remains to be seen whether wrangles over budget problems and the high price of European grain will allow the heads of government time to put flesh on the bones of Mitterrand's dream. The big danger is that ill-considered enthusiasm will point this important enterprise in the wrong direction.

Here is a cautionary tale. While very little has been said of the way in which Eureka would be put into effect, one theme of the past few weeks has been that European governments should settle on a few major technical objectives and then pool resources so as to make them come to pass. What kinds of projects? On the face of things, a scheme to equip the air forces of Western Europe with a military aircraft designed to take over from those now in service would seem to be an obvious (if unambitious) candidate. As it happens, this is a scheme on which five European governments (Britain, France, Italy, Spain and West Germany) have already embarked. So what are the enthusiasts for Eureka to make of the meeting last week in London, when the governments concerned failed to agree on the specification of the aircraft they would jointly build, and on a scheme for sharing the construction work among themselves? That does not promise well for the simpler goal-oriented versions of Eureka, although there is a final meeting next month, at which something may be salvaged.

The lesson from last week's meeting is that it will make no sense for European governments to invest in the improvement of technology if they do not at the outset agree on the partition of the benefits. The temptation will be to attempt to divide the spoils in proportion to the investments made by the several governments, but attempts to legislate for chauvinistic equity along these lines are doomed to failure. Xenophobia being what it is, even within the European Communities, at least one but most probably all partners would finish with a sense of having been short-changed. The other more logical basis for mutual

support for technological developments would be to say that the benefits of the investments proposed could be exploited by whichever European companies first had the wit to recognize their utility. In the long run, Europe as an economic entity would benefit, but some parts of it more than others. Equity would then require that there should be mechanisms for redistributing wealth among European states, which amounts to saying that Europe should function as a federated state. Politically that is a long way from reality. If Eureka is to succeed this side of infinity, there will have to be some halfway house.

Much will depend on the kinds of projects on the agenda. So far, there have been only general statements of what might be done. People such as President Mitterrand have been talking of the further development of integrated semiconductors, of machines with artificial intelligence and, more recently, of the exploration of the deep oceans. On one view, the particular choice of projects to support is almost immaterial. Provided that the objective is sufficiently ambitious, the enthusiasts say, and Europe's engineers sufficiently committed, there will be a host of incidental technical developments which cannot fail to benefit European industry as a whole. This is the argument by which the 1960s Apollo programme to send people to the Moon was justified not by its ostensible objectives but by its technological spin-off. What this week's summit should remember is that such arguments are an assault on reason and a recipe for extravagance. If the technologies of, say, welding and refrigeration need to be improved, why not mount market-oriented developments in those fields? Especially when one of Europe's problems is a shortage of skill, the consequences of too much ambition could be a damaging diversion of resources.

Eureka should begin from a careful analysis of why Europe fails at present. The most obvious weakness is that Europe is not yet a common market. One result is that companies cannot accurately tell what research and development is likely to meet market needs. Another is that there are too many small companies duplicating each other's efforts. Eureka needs a way of circumventing these constitutional obstacles, perhaps by allowing separate companies to collaborate in transnational research and development partnerships that could be blessed with common funds. To work on what? The heads of government at Milan will no doubt be flush with ideas. They should keep them to themselves. Politicians and civil servants have a poor record as entrepreneurs. What is needed is a mechanism by which groups of companies can put forward detailed proposals as if they were applying for research grants, but with the difference that the criteria for success would include not merely the novelty and the feasibility of the proposals, but some estimate of their economic and strategic promise. The European Commission's recent suggestion that the Euratom treaty provides a framework for channelling common funds into novel enterprises would make sense in this connection.

This week's summit must also give some thought to the infrastructure. One of the reasons why Europe's technology lags behind that of the United States and Japan is that its technological labour-force is ill-provided with first-hand demonstrations that the future can be made to happen here and now. Part of the trouble is that higher education is run on a shoestring (Britain) or is flexible (France and West Germany). Spending more on

Biological manuscripts

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the university education of technical people is not a sufficient answer, although some of that will be needed. More to the point is the need that Europe, in trying to create the foundations for industrial prosperity in the next century, should find some way of enabling the now-young people who will inhabit that world to make their full mark. In many ways, Europe has grown too old.

The third need for the summit is to remember Adam Smith. Inward-looking Europeans see their enterprise as a self-contained imitation of what has already happened elsewhere. Too many Europeans consider that the immediate goal must be to replicate what IBM and AT&T have done in the United States, or what a host of Japanese companies have done in consumer electronics. The fallacy is that success would merely bring the prospect of head-on competition in fields that are certain to seem old-fashioned even a decade from now. But there is no reason why the world should become less interdependent than it is already, for which reason the trick for Europe must be that prosperity should rest on industrial activities complementary to those elsewhere. That is why the objective of those who endorse Eureka should be to build on Europe's present strengths, such as they are, not simply to imitate what others do. The goal must be an economic and economical division of labour, to which end originality is the crying need. Will the summit have the courage to recognize that simple but daunting truth? □

How many (what) miles?

Last week's shuttle flight shows that the Strategic Defense Initiative has a lot to learn.

THE timing of last week's fiasco in which a laser beam from Hawaii was directed at the wrong side of the US shuttle in orbit about the Earth could hardly have been worse. The US Congress is at the stage in the annual budget cycle when critics of the Strategic Defense Initiative (SDI) are energetically — and successfully — chipping away at the huge budget the administration has asked for. While nothing is settled, the chances are that the budget for the financial year beginning on 1 October will be reduced by between a quarter (according to the Senate) and a third (according to the House of Representatives) from the \$3,700 million asked for. If the managers of SDI are wise, they will spend at least some of that sum on teaching each other a little about elementary goeodesy.

The official explanation of the first failure of the laser test (which worked on later orbits) is quite breathtaking in its innocence. Almost merrily, the spokespeople have been saying that, by some oversight, the "on-board" computer on the shuttle had been programmed "in" statute rather than nautical miles. By their nudges and winks, they have implied that only some profound perversity can explain the habit that has people who travel in shuttles measuring distance in the maritime measure of nautical miles. No doubt SDI officials will have figured out that nautical implies something to do with the sea, because *Nautilus* is a kind of submarine. In their innocence, they seem not to know that a nautical mile is intended to be the length of a minute of arc along a great circle on the surface of the Earth, although it was prematurely defined as 6,080 feet by the British Admiralty in the eighteenth century.

The SDI people are unlikely to be too downcast by this show of ignorance; they have earned a reputation for being brash. They have a tendency, when they encounter physical problems that would seem insuperable obstacles to others, to make a solution of them a "requirement" of the SDI programme as a whole. The little confusion between statute and nautical miles may, in any case, make them seek safety in unambiguously named kilometres instead (a cause too long dormant in the United States). So perhaps they should be told that the French Revolution had a committee, of which both Laplace and Lagrange were members, which defined the kilometre in much the same way as the nautical miles as one ten-thousandth of the distance between the Equator and the pole. □

Laser proliferation

The US Department of Energy thinks the laser enrichment of uranium feasible; that is bad news.

THE third review conference of the non-proliferation treaty (NPT), due to open at Geneva in August, will be forced to reckon with a late agenda item, the recognition that the simplest way of making nuclear weapons is probably now the enrichment of uranium by means of lasers. That is the implication of what appears to have been a successful demonstration of laser enrichment at the US Lawrence Livermore National Laboratory in California. The laboratory has been able to make five kilograms of uranium-235 in a single purification step. For those who wish to make bombs, the advantages of this route are obvious. Unlike isotope separation by gaseous diffusion or centrifugation, laser separation may be a once-through process. So capital costs are likely to be less and, for potential violators of agreements, separation plants will be inconspicuous.

None of this implies that uranium enrichment has become child's play. At Los Alamos as well as at Livermore, people have been trying for at least a decade to distinguish by means of lasers between the different isotopes of particular elements, with uranium usually at the backs of their minds. The fact that success has eluded them for so long is one comfort, suggesting that governments will not be able to run up material for a few bombs overnight by buying standard equipment from commercial suppliers. Indeed, the technology of copper excimer lasers reported (see p.706) to be the primary energy source for the Livermore process will not easily be replicated elsewhere, at least for the time being. In any case, crucial details such as the identity of the electronic transitions by means of which uranium atoms are excited to near-ionization are sensibly being kept secret. The obvious snag is that uranium atoms are the same the whole world over, and that the investigation of the spectroscopy of uranium required to specify the ingredients of a laser enrichment machine should be little more than few months' work for a couple of competent physicists. It is a fair guess that even governments with no interest in making bombs, but with an interest in what others may be doing, will put this work in hand immediately. Putative bomb-makers will probably be even quicker off the mark.

So does this new development mean that there is no longer any purpose served by attempts to control the spread of nuclear weapons by the physical control of fissile material? Fortunately, not quite. NPT is a compact between non-nuclear governments and the nuclear powers under which the first group undertakes not to make nuclear weapons, and to put up with physical inspection by the International Atomic Energy Agency, while the second undertakes not to help others make bombs but to help with civil nuclear technology, and at the same time to negotiate measures of arms control that will make the world a safer place. The signatory nuclear powers (France and China have not joined NPT) will be given another drubbing at this year's review conference for having done so little on arms control, but it seems unlikely that non-nuclear powers will desert the treaty on that account, or because laser enrichment seems to offer rivals outside the treaty a more convenient route to bomb-making. Keeping safeguards will be more than ever a demonstration of self denial.

The more serious problems are what they have always been, the abstention from NPT of countries which believe they may gain from being thought potential nuclear powers — Israel, Pakistan and so on. If laser enrichment means anything, it is that governments within NPT should have a powerful incentive not merely to control the use made of fissile material but also to see that the international trade in uranium is also well-regulated. For however convenient the new technique may be, it will still take the best part of a tonne of uranium to extract a critical mass of uranium-235, which should be a noticeable amount of such a scarce material. That is the direction in which the interests of the regulators should now be shifted. □

European research

Milan the spur to new collaborations?

Brussels

THE European Commission sees this week's summit meeting at Milan, where the French Eureka proposal is certain to have at least a brief hearing, as an opportunity for persuading the members of the European Communities to take a more serious interest in cooperative research and development. But Mr Jacques Delors, president of the Commission, and his technology commissioner, Karl Heinz Narjes, are keeping an open mind on how collaboration might develop.

Delors wants flexibility above all, for which reason he is keeping up his sleeve several different legal frameworks for technological collaboration. One possibility is that the existing Euratom treaty might suffice, another that there might be a new treaty along similar lines. But that, everybody agrees, would take too much time. The Commission seems to be saying that it merely wants the member states to agree to collaborate, leaving the details of collaboration to be worked out later.

According to Delors, money is all important. He insists that there should be a "critical mass" of funds for research and development. Part of his ambition is that member states should come to regard common spending on research as the European equivalent of US spending on military developments, where commercial beneficiaries are not expected to repay the public investment.

One mark of members' commitment will be whether they agree to the 14.9 per cent increases of the Communities' research budget marked out for 1986. Narjes believes that research and development will have to take a larger slice of the Communities' budget in future years, perhaps between 6 and 8 per cent of the total by 1990. Whether the member states will happily accept the implication of increased budget contributions to the Community is another matter.

Responses so far from member governments have been mixed, according to Narjes, who sees European initiatives in research and development as essentially collaboration on projects to which industrial companies would contribute, as in existing collaborations. While some governments are concerned at the prospect of Community control of common projects, the Commission notes that British companies have shown more interest than their government in the proposed collaborative programme, Research in Advanced Communications in Europe (RACE).

In its optimism, the Commission does not expect that its plans to expand col-

laborative research will founder on the expectation of member states of a return proportional to their individual investments. But changes will be needed in what governments expect. And there will also have to be more decisive steps towards the creation of a Europe-wide system of standards and a greater degree of cohesion so that it would be more difficult than at present for competitors elsewhere to "pick off" European states one by one.

Narjes does not expect more from this week's summit than agreement among the member states to settle on two or three projects for further collaboration by the end of 1985. He has a shopping list of areas in which collaboration might be sought,

ranging from large computers and superchips to high-speed trains and biomolecular engineering to particle beam physics and optical communication. The Commission would like to see the French Eureka project subsumed in an agreement that Europe's collaborative research programmes should be expanded.

Pragmatically, the Commission says that the legal forms of novel collaborations will be decided only after specific programmes have been agreed. One obstacle ahead, according to Delors, is that Europe's technical labour force is ageing more quickly than that of the United States and Japan. The Commission's ambitious plans are in part intended to be a spur to the training of more skilled technologists, if only because partners in the new collaborations that may come into being will know that they will benefit only in proportion to the numbers of people able to help exploit the results of research and development programmes.

Anna Lubinska

Chemical weapons

US moratorium to end

Washington

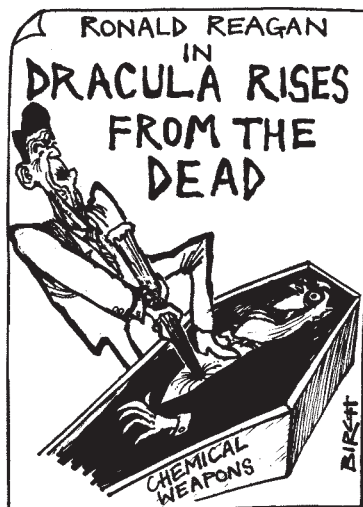
THE US House of Representatives voted last week to end a 16-year moratorium on the production of chemical weapons. Actual production of the new "binary" weapons, which contain two relatively non-lethal chemicals that are mixed together in flight to produce nerve agent, could begin in December 1987.

The Reagan administration, arguing that current stockpiles of nerve-gas shells

sions, however. Funds for production would not be released until 1987, and only after the President certifies that the weapons are needed. Supporters also accepted an amendment that would require the NATO (North Atlantic Treaty Organization) allies to declare their willingness to have the US weapons deployed in their countries before production could begin. However, even opponents of the new weapons expect that amendment to be dropped by a House/Senate conference which must meet to reconcile differences between the two houses' bills. Congress will still have to agree to appropriate the funds for production.

The House bill authorizes an initial expenditure of \$124 million; the Senate bill, \$163 million. Critics have said that the total cost of carrying out the Pentagon's production plan could exceed \$4,000 million.

Stephen Budiansky



are deteriorating and do not represent a "credible" deterrent, had repeatedly sought congressional approval for the new weapons over the past three years. In close votes, the House had repeatedly rebuffed the President. This time, however, heavy White House lobbying paid off. The Republican-controlled Senate had already approved the proposal.

Supporters did make several conces-

Nature in Japan

NATURE announces an international conference to be held in Tokyo next year, on 22-24 January 1986, on the theme *Molecular biology becomes biotechnology*. Speakers at the conference will include ten from the United States and Europe and an equal number from Japan. The conference, which will interest molecular biologists in academic and industrial laboratories, is in the series which includes this week's conference in London and that, in conjunction with the University of California, San Francisco, to be held in San Francisco next October. Further details may be had from *Nature's* offices in London and Tokyo. □

Auto emissions

Europe nears compromise

Brussels

Too tough for some, too lenient for others, the Commission of the European Economic Communities (EEC) was last week required to defend its proposals to limit car exhaust pollution. At the same time, its experts began a last minute search for a compromise to avert an otherwise inevitable deadlock when the ten environment ministers meet in Luxembourg on 28 June. The compromise could involve not only a possible redefinition of the troublesome medium-range category of car (now set at 1.4–2.0 litres) but also different emission standards for cars fitted with three-way catalytic converters and those relying on lean-burn technology.

Reactions to the original proposals (see *Nature* 20 June, p.622) have been sharply divided among the member states. France, Italy and Belgium have joined the United Kingdom in dismissing the emission standards for medium-sized cars as too strict. But West Germany, followed by the Netherlands, Luxembourg and Denmark (which wants to see US-style uniformly strict controls applied to all categories of cars), is set on tougher controls. In addition, the Commission's proposals have reopened the debate on large cars (2 litres and over) that appeared

to have been settled in principle on 20 March. The figures put forward by the Commission are unacceptable to Belgium, France and the United Kingdom despite the fact that the use of the three-way converter in the case of large cars had been agreed in March.

The Commission's proposals have also been sharply criticized by an outside expert, Dr Michael P. Walsh, former deputy assistant administrator for the US Environmental Protection Agency's mobile source air pollution control. In two papers made public last week, he maintained that the Commission's proposals ignore a number of important factors. In particular, they do not take account of increased car sales which, given the long timetable for implementing the proposals, could mean "significantly more pollution both in the short and long term".

According to Walsh, by the time the entire fleet is converted to the new standards, nitrogen oxide and hydrocarbon emissions will be almost three times more than equivalent US levels and carbon monoxide almost two and a half times more. "If member states choose to implement more 'permissive' standards, these estimates", he says, "are optimistic."

A major part of the problem is the test cycle used to establish the amount of pollutant emitted. The European test cycle, says Walsh, takes little account of modern driving conditions. A new European test cycle is being run at present, on the basis of which the standards now to be agreed will be modified in a year or two. This, Walsh says, will only serve to complicate the matter of laying down US-equivalent standards now.

The European Commission has always insisted that it is impossible to apply the US cycle to European cars. But Walsh says that European studies have shown that US test procedures are a more faithful reflection of current urban driving conditions. He concedes that the European test is effective in simulating dense congested traffic conditions, but says it will not suffice for assessing limits for NOX and hydrocarbons (which are generated at high speeds and carried over long distances).

Walsh maintains that the Commission's proposals as they stand will cut NOX emissions by only 39 per cent by the year 2010, while HC and CO emissions will fall by 24.4 per cent and 22.8 per cent. On the other hand, if US equivalent standards in the range of 4.6 grams per test HC plus NOX, 16 g/t CO and 2.4 g/t NOX were applied for medium and large cars, the actual reduction in pollution could be 66.7 per cent for CO, 75 per cent for HC and 76.7 per cent for NOX.

In response, the Commission has produced a paper supporting its proposed standards, which it says will reduce all car exhaust emissions by 70 per cent in the case of large cars and 73 per cent for medium cars, compared with 67 per cent reduction reached in the United States. Emissions of NOX, which are a principle concern of EEC, would be reduced to a level only slightly above that in the United States (1.56 million tonnes each year in EEC, compared with 1.48 million tonnes in the United States) with a further reduction possible when new limits (perhaps 5 g/t) are introduced during the planned second phase for small cars under 1.4 litres, bringing EEC emissions down to 1.41 million tonnes.

Commission experts estimate further that if EEC ministers agree to introduce US-type speed limits of 55 m.p.h. (88 k.p.h.) as well, global NOX emissions from Community cars could fall to 15 per cent below US levels. But this suggestion has so far been blocked by West Germany, whose cars incidentally emit the greatest volume of NOX (637,000 tonnes in 1980) and which is under the greatest pressure to adopt tough controls on air pollution.

Anna Lubinska

Mount Wilson's end

Washington

No rescue plan has materialized to save the Mt Wilson 100-inch telescope, due to be shut down next week by its owner, the Carnegie Institution of Washington (CIW). Although discussions are continuing to find a "suitable operator" to take over the facility, it seems increasingly unlikely that one will be found. The several rescue groups that had been formed by interested astronomers, professional and amateur, have apparently failed to raise the several million dollars necessary to keep the telescope in operation.

Carnegie announced its intention to close the telescope a year ago, citing the increasing financial burden of running both Mt Wilson and its newer Las Campanas observatory in Chile. More recently, CIW revealed plans for shutting down the other astronomical facilities at Mt Wilson. The 60-inch reflector, now used for solar and stellar observations, will continue operating until 1 July 1986. The solar observatories will continue operating only until next October, but CIW is willing, until 1 July 1986, to continue administering salaries and other operational budgets provided by outside grants. In other words, CIW offers a nine-month lease to outside investigators who can find their own money.

Stephen Budiansky

Verification in space

Los Alamos, New Mexico

DONALD Kerr, director of Los Alamos National Laboratory, is calling for the United States to give more attention to how it would detect and assess new high-technology Soviet anti-missile defences — in other words, a Soviet star wars system. Kerr says that "the possibility should be anticipated" that the United States and Soviet Union might reach agreement to limit star wars defences, and points out that the first requirement of any such agreement would be a means of verifying compliance. Conventional reconnaissance might not be adequate to assess the capabilities of a Soviet star wars system.

Despite the new technical challenges of such a monitoring system, Kerr says he is "moving in that direction". The testing of laser weapons might be detected by looking for atmospherically-scattered repetitive pulses of light, for example, and satellites might be interrogated with particle beams to see if they contained fissile material: the radiation emitted would provide information on the isotopes present.

Kerr plans to argue his case in Stockholm next month at a conference on space weapons and international security hosted by the Stockholm International Peace Research Institute. Los Alamos National Laboratory has long undertaken research into ballistic missile defence and claims to be well placed to develop means of verifying compliance with anti-ballistic missile or other future treaties limiting arms in space.

Tim Beardsley

Blood typing

US sale of British reagents

BLOOD-GROUP typing in the United States is likely to be based on monoclonal antibodies, following a deal between Celltech, the British biotechnology company, and Ortho Diagnostic Systems Inc., the Johnson & Johnson subsidiary which is the largest supplier of blood-typing reagents in the United States.

Although the British National Health Service has already switched over to Celltech's monoclonals for ABO blood typing, the technology was not simply transferable to Ortho. The US blood-typing test is differently and more rapidly performed than the British test. Moreover, Ortho's blood-typing reagents, based on antibodies from donated blood, are designed to detect blood group antigens at below the threshold at which British tests operate.

These demands have required a two-year period of development by Celltech, with support from Ortho. Because the single monoclonal antibodies against blood groups A and B developed for use in Britain did not perform adequately in the US tests, Celltech embarked on a worldwide hunt for more appropriate antibodies. Despite screening hundreds of candidates, it failed to find sufficiently sensitive single A and B antibodies. So

Ortho's reagents are each a cocktail of several monoclonals, some of which Celltech have had to buy in from outside. At least one unnamed US academic laboratory stands to gain.

There have also been regulatory hurdles to cross. Because traditional blood-typing reagents are blood products, they are licensed by the Office of Biologics of the US Food and Drug Administration. The monoclonals, although derived from cultured cells rather than blood, have been caught by tradition and subjected to the regulations of the Office of Biologics, although other diagnostic monoclonals have passed through other offices.

Material is at present produced in 100-litre fermenters, but as Ortho's demand builds up, Celltech plans to switch to its 1,000-litre fermenter which can yield 100 grams of antibody in a two-week run. If, as expected, monoclonals are eventually used in half of ABO blood-typing tests worldwide, "a low number of kilograms" of antibody will be needed annually. According to Gerard Fairtlough, chief executive of Celltech, independent estimates put the world market for blood-typing reagents at £7.5 million per year. The initial agreement between Celltech and Ortho is for five years, involving

payments of "several million pounds" to Celltech by Ortho.

John Berriman of Celltech sees no obvious competitors. One Canadian and one Swedish company have potentially suitable monoclonals, but not Celltech's ability to produce them in quantity. Celltech also hopes to produce a monoclonal antibody for rhesus blood typing, but because mice do not produce antibodies against the human rhesus antigen, it has not been possible to use standard hybridoma technology. Instead, Celltech hopes to develop a system of producing a monoclonal antibody against D antigen, the main rhesus antigen, in human lymphocytes transformed by Epstein-Barr virus.

Peter Newmark

AIDS

France to screen blood donors

FRANCE could begin testing all blood donors for antibodies to the virus causing AIDS (acquired immune deficiency syndrome) "within a week", and donors may be told the results. This dramatic action to limit the spread of AIDS in France is pre-empted by the announcement by the Prime Minister, Laurent Fabius, in the National Assembly last week that, "after much reflection", he had decided that a test must be "obligatory".

The tests will be carried out by the blood transfusion service SNTS (Service Nationale de Transfusion Sanguine), which has been evaluating three different commercially available tests. One is French, prepared by the Institut Pasteur and marketed by the drug company Sanofi, and two US — one from Abbott Industries and the other from Electro-Nucleonics (distributed in Europe by the Dutch company Organon). All three tests are based on the ELISA technique (enzyme-linked immunosorbent assay) using killed virus. Because of the loss of envelope protein during the preparation, all of them are more sensitive to antibody to the virus inner protein (*gag*) than to the envelope protein antibodies.

Exactly which test will be selected is not for SNTS to decide, but Fabius spoke favourably last week of the French product, "which could be exported throughout the whole world". SNTS say that tests could begin within a week, although Fabius himself did not announce a date. He did, however, indicate a cost — FF 200 million (£20 million) a year.

On the question of informing donors if AIDS antibody is detected, Fabius is not yet convinced. He has asked for a "more detailed" analysis, and has set up a committee of experts to report to him as soon as possible. The tests are necessary, but unnecessary fears should be avoided, he said.

Robert Walgate

Searle deals blow to Genex

Washington

GENEX Corporation, which had pinned its hopes for making an early profit on mass production of the chemical L-phenylalanine, the key starting material for the artificial sweetener aspartame, has been dealt a major setback. G. D. Searle, which holds exclusive rights in the United States and most of Europe for the use of aspartame as a sweetener, last week informed Genex that it would be dropping its contract to purchase phenylalanine as from November. Genex is left with an \$18 million plant that the company says it built on assurance from Searle that it would continue to be a major supplier. The plant, located in Paducah, Kentucky, was completed only last year and it reached full production three months ago.

Genex's president, J. Leslie Glick, acknowledges that the market for other uses of phenylalanine, mainly the production of pharmaceuticals, is "limited", and could not justify keeping the Paducah plant open.

Genex last year had sales of \$20.6 million, almost all of that from phenylalanine. Research and development contracts, which totalled \$5.7 million last year, will now once again become the company's major source of revenue. Glick said the company was looking into the possibility of using the Paducah plant for enzyme pro-

duction or for joint ventures. Only 25 per cent of the facility is now used for phenylalanine production. The company had been reserving the remaining space for expansion in other areas.

Glick says that Genex's loss for 1985 would be less than last year's despite the setback, largely because the high cost of starting up the Paducah plant was included in last year's accounts. Searle refuses to comment on the decision not to renew its contract with Genex. Searle is known to have expanded its own capacity for producing phenylalanine and it also purchases an undisclosed quantity from Ajinomoto in Japan. Genex's process involved an enzyme reaction of cinnamic acid. Ajinomoto (and Searle, which licenses the process from Ajinomoto) uses selected strains of bacteria and fungi that produce large amounts of phenylalanine from sugar.

Searle patents on the use of aspartame expire in the United States in 1992 and in Europe beginning in 1987. Genex's plant is producing about 2,000 metric tons of phenylalanine a year, about two-thirds of Searle's current need, according to independent estimates. It is estimated that by 1987 the demand for phenylalanine will be 5,700 metric tons per year. Searle's sales of aspartame last year were worth a total of \$585 million.

Stephen Budiansky

Thermonuclear fusion

Inertial confinement in trouble

Washington

ANTARES, the world's most powerful fusion research laser, could face premature retirement later this year after only two years in operation, unless the US Congress takes pity on the \$63.5 million instrument. The laser, at Los Alamos National Laboratory, is being used for experiments in inertial confinement fusion, but has proved to be the instrument of its own fate: it has clearly demonstrated that carbon dioxide lasers such as Antares have no potential for economic fusion. Hopes of a reprieve are now pinned on the possible use of Antares in the Strategic Defense Initiative (SDI) programme of research into anti-missile defences.

Antares is capable of producing a 40-kilojoule pulse of light lasting for one nanosecond. The problem is that radiation of wavelength 10.3 micrometres is, with the benefit of hindsight, too long for

economic fusion power. When focused onto a glass bead containing fusion fuel, radiation of this wavelength stimulates the emission of hot electrons that pre-heat the fuel and so prevent the high degree of compression and simultaneous high temperature needed to achieve plasma ignition. Carbon dioxide lasers would be useful only if their cost dropped drastically, to about \$10 per joule. This is an unlikely prospect; Antares costs \$1,500 per joule, and the cheapest imaginable mass-produced carbon dioxide laser would still cost \$300 per joule.

The administration's inertial confinement fusion budget request for fiscal year 1986 assumes that Antares will be taken out of service before the end of 1985. The total request at \$70 million is about the figure for the current year. Donald Kerr, director at Los Alamos, has testified before Congress that about 100 people may

lose their jobs in inertial confinement fusion if the proposed cuts go ahead.

David Cartwright, principal programme manager for inertial confinement fusion at Los Alamos, has recently informally requested an extra \$5 million to allow the current series of experiments on Antares to be completed, because recent results suggest that the temperature of the troublesome hot electrons can be reduced by increasing pulse duration from about 1 nanosecond to 5 nanoseconds. This sum would nevertheless keep Antares in operation for only a few months, and would not allow modifications for new experiments. Hence the hopes that the SDI organization might take an interest.

Cartwright says Antares is the only national facility that could be used to test the predicted effects of high intensity laser radiation on nuclear warhead re-entry vehicles. The pulse length and power could without much difficulty be increased to a few microseconds and 0.25 megajoules, a region of importance for laser weapon concepts. Mirrors would have to be repositioned so that, instead of converging in three dimensions on a single point, the laser beams would illuminate a larger spot from one side. A request for \$15 million has been made to the SDI organization for this purpose. Antares might also be used to drive X-ray lasers, also of SDI interest.

Congress is sympathetic to the idea of keeping Antares running, and both houses have authorized sums of around \$145 million for inertial confinement fusion — twice the administration's request. The appropriations hearings, which will decide how much will actually be spent, have, however, only just started.

Some support for Antares has come from the National Academy of Sciences, which is carrying out for Congress a study of inertial confinement fusion. The committee responsible has made known its opinion that it would be unwise to dismantle a facility while its usefulness is still being evaluated. Congress might therefore take this cue to override the Office of Management and Budget, as it has been known to do before. Congressional aides warn, however, that there may be jurisdictional obstacles to supporting Antares directly through the SDI organization, if only because that would entail a transfer of funds between two subcommittees. On Capitol Hill, subcommittees' territories are guarded jealously, and the political difficulties are seen as far more daunting than the technical modifications that Antares itself would need.

The other major fusion research laser facility in the United States, known as Nova, is close to completion at Lawrence Livermore laboratory. The light produced by the neodymium-glass lasers in Nova has a wavelength of around 1 micrometre, but is then frequency-multiplied by a factor of 3 to give light with 0.35 micrometre wavelength. Even this is not expected to

Uranium enrichment

France also plumps for lasers

THE French nuclear fuel company, COGEMA, announced earlier this month plans to enrich uranium by laser, rather than by the diffusion or centrifugation of uranium hexafluoride gas, and said that this would cut costs by a factor of two. But one French observer says the statement is a "bluff" prompted by competition with the United States for the supply of enriched uranium to Japan and by a wish to pre-empt the announcement by the US Department of Energy of its decision to adopt the laser process.

Research on laser enrichment has been under way at the laboratory of the Commissariat à l'Energie Atomique at Saclay, near Paris, for some years. But the feeling in France appears to have been that the technique was not yet required, so that the project has been poorly supported. According to M. Claude Benaud, director of the isotope separation research unit at Saclay, it will be 15 years before full-scale laser separation plants are in operation.

Benaud spends some FF 100 million (about £9 million) a year on laser separation experiments with the French SILVA process, which is similar to the AVLIS process, developed by Lawrence Livermore Laboratory in the United States, using uranium metal vapour. But following the COGEMA announcement, he expects his budget to rise next year by some 20–30 per cent, taking SILVA to the "pre-pilot phase". The separation of kilogram quantities of uranium will come only later, Benaud says. The Lawrence Livermore Laboratory, after a year's frantic competition with the Oak Ridge advanced gas cen-

trifuge, has now claimed the successful enrichment of some five kilogrammes of uranium.

Even so, says Benaud, there is no commercial need for a laser plant as yet. So why the US haste? One British laser physicist believes the spur may be the need to accumulate separated plutonium for the increasing numbers of US multi-warhead (MIRV-ed) missiles. In these missiles space is at a premium, and plutonium, with a lower critical mass than uranium, makes smaller triggers. The United States has plenty of plutonium in spent fuel from its 90-odd pressurized water reactors, but that is contaminated with isotopes which dampen the fast chain reaction which is essential in warhead applications.

AVLIS, or SILVA, could help. The principles could be applied to any isotope system, but Benaud says he is not at liberty to say if his group has worked on plutonium.

For commercial uranium separation, on the other hand, French sources are more forthcoming. Estimates are that the optimum size of a laser plant will be some ½–1 million units "separative work" (SWU) a year, enough to supply three to six 1,000-MW pressurized water reactors with fuel. By comparison, the giant Eurodif diffusion plant at Tricastin in France is capable of nearly 11 million SWU a year, but is under-utilized. Laser separation will be cheaper in the long run, according to Benaud. Indeed, COGEMA is said to have offered in Japan to halve future enriched uranium prices.

Robert Walgate

be economically viable for fusion; the best hope among lasers is probably the krypton fluoride excimer laser, which has a wavelength of 0.25 micrometres and which has been developed since work on Antares and Nova began. Los Alamos has a small experimental krypton fluoride laser known as Aurora. Many now believe, however, that light ion beams will beat lasers to achieving plasma ignition. What might be the first machine to reach plasma ignition, known as Particle Beam Fusion Accelerator-2, will begin tests early next year at Sandia National Laboratory in New Mexico.

Tim Beardsley

Proliferation

THE atomic vapour laser isotope separation process (AVLIS), chosen by the US Department of Energy for separating the fissile uranium-235 isotope from natural uranium (see above), relies on selective three-photon ionization in an electric field, which sweeps out the ionized uranium-235.

Success depends on the relatively large (1 GHz) isotope shifts of spectral lines of the actinide elements on the low thermal velocities of such heavy atoms (implying reduced Doppler broadening) and the use of laser beams impinging perpendicularly on a collimated jet of uranium vapour (reducing Doppler effect still further).

In principle, the excitation could be effected in two steps, by a photon carefully tuned to an internal excitation and a photon of almost any wavelength to take the excited electron out to the continuum, leaving the atom ionized. But the ionization energy of uranium-235 is so high that the two photons would have to be at the blue end of the spectrum, where tunable lasers are expensive and where photons tend to degrade laser mirrors. So AVLIS uses instead two low-energy mid-spectrum photons, produced by a dye laser, to effect the finely-tuned selective excitation. A third ultraviolet photon is then used to take the excited electron far out into the continuum, where auto-ionization resonance increases the ionization cross-section tenfold.

The use of two successive excitation photons also has the advantage that each can be fired from different sides of the uranium jet, so that the first-order Doppler effects cancel. The Lawrence Livermore dye lasers are pumped by a high-efficiency high repetition-rate copper-vapour laser which has the advantage over continuous lasers of an extremely high average power.

According to one British laser physicist, such devices "could be run in a university laboratory" to separate kilogram quantities of fissile uranium-235. If a dye-laser system were established in Libya, for example, "it would be invisible", which is "why we're worried about them".

Robert Walgate

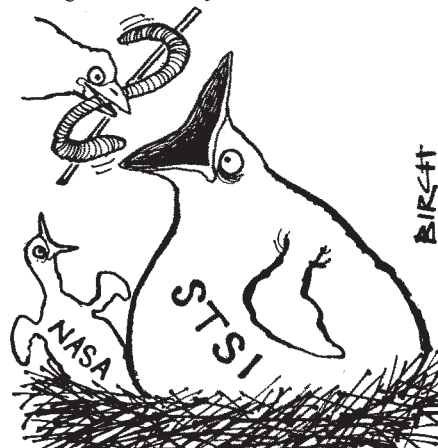
Hubble telescope

Space institute's wings clipped

Washington

A STUDY of the Space Telescope Science Institute (STSI) by a National Research Council task group has concluded that the ever-expanding institute should not be allowed to grow much beyond its present size. The study, which was commissioned by the National Aeronautics and Space Administration (NASA), says that new scientific functions required for the Hubble Space Telescope should instead be contracted to outside organizations, with STSI restricted to an advisory role wherever possible.

The task group undertook its study in the light of widespread concern in the



astronomy community about the institute's rapid growth since it was established in 1981. NASA originally planned an institute with a staff of 100 and an annual budget of less than \$5 million; STSI's budget for this year, however, is \$12.8 million and is expected to reach \$21 million by the end of the decade (at 1984 prices). The staff already numbers over 200 and is expected to top 250 before long. Some fear that the institute might squeeze out other NASA astronomy projects.

The task group, chaired by William Gordon of Rice University, agrees that NASA's original idea of having a special institute to manage science for the space telescope was a good one, and says the institute, which is based at Johns Hopkins University, has done a good job of surmounting the many unplanned technical obstacles that have beset the space telescope. But the task group euphemistically suggests that STSI's stated objectives should be modified by adding a requirement that STSI work "within available resources". What those available resources should be is not specified, although the task group notes that STSI was intended to be comparable in size with other ground-based astronomy facilities; that being the case, STSI is already close to the upper limit.

Gordon's task group also has some implied criticism for the Association of Universities for Research in Astronomy

(AURA), the organization that is nominally in control of STSI. Gordon says that AURA should play a more active role in mediating in the frequent jurisdictional conflicts that have arisen between NASA and STSI.

Other recommendations include the suggestion that STSI develop remotely accessible data archives and software that can be run on computers at researchers' home institutions, to avoid pressure on computer time at STSI. The task group also says NASA and STSI should plan a joint public information service for when the telescope is operational. In the longer term, however, Gordon says it is up to NASA to make clear its plan for the future of the institute.

Tim Beardsley

The report, Institutional Arrangements for the Space Telescope: A Mid-Term Review is by the Committee on Space, Astronomy and Astrophysics, National Research Council, Washington, DC.

East German physicist

Absence noted

At a physics conference in Visby, Sweden, this month, Dr John F. Sharpey-Schafer from the University of Liverpool announced that his paper was dedicated to Dr Stefan Frauendorf of the Zentral Institut für Kernforschung at Rossendorf, East Germany. Dr Frauendorf had been invited to read a paper at the conference, but was arrested last November, and is now said to be facing charges of espionage, based on alleged contacts with West German intelligence services.

Dr Frauendorf had for several years been a regular visitor to the Niels Bohr Institute in Copenhagen, where he was working with several other scientists on a joint project on nuclear structure.

At the time of his arrest, he was in the process of reading and correcting a paper, written jointly with the Niels Bohr team, on high spin states. This paper seems to have disappeared at the time of his arrest and, as there was no copy, much work has had to be redone. A letter from the Niels Bohr Institute, enquiring about the paper and deploring the breaking off of collaboration with Dr Frauendorf, brought only the answer that the physicist was under investigation for espionage carried out over a number of years.

The Niels Bohr Institute has been working with Rossendorf for some 20 years, an association which has been especially fruitful over the past five years or so, roughly the period of Dr Frauendorf's visits. Cooperation arrangements have not been entirely broken off by the incident, however; three further visitors have arrived from Rossendorf since the scientist's arrest.

Vera Rich

Venus

Soviet probes make soft landing

THE first part of the Soviet Veba (Venus-Halley) mission, the descent of atmospheric and surface probes to Venus, has been a success, according to Dr Vladimir Kotel'nikov, first vice-president of the Soviet Academy of Sciences. Indeed, in good Soviet style, the Veba-2 surface probe, which included a rock-gathering minidrill and spectrometer analysis chamber, overfulfilled its norm, working and transmitting data for 22 minutes and 17 seconds rather than for the expected 14 minutes. "Express analysis" of incoming data, moreover, revealed a number of new results, both confirming previous postulates and producing various surprises.

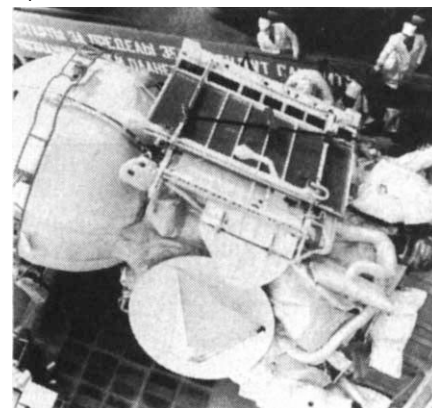
The Veba-2 descent module, which landed in the Rusalka plain ($6^{\circ}27'$ S. latitude, $181^{\circ}5'$ longitude), has revealed the presence at that point of soft sandy soil. Previous missions had revealed a more rocky terrain and, although several planetary podologists had postulated that more friable soil might be encountered, this is the first direct evidence of them. Similarly, the two drifting aerostatic probes (carried by helium balloons) directly confirmed the presence of large quantities of sulphuric acid in the venusian clouds. These two probes drifted around the planet's equator for a quarter of its circumference, with a translation velocity of 60 to 70 metres a second. Surprisingly, the balloons encountered updraughts and

downdraughts of up to 1 m a second, which hurled them up and down by 200 to 300 m. The drift data from the probes, which were tracked by radio telescopes at Medvezh'i Ozera and Ussriisk in the Soviet Union, Pentikton (Canada), Fort Davis (United States), Madrid, and Parkes (Australia), will be processed in conjunction with the CNES centre at Toulouse. Full results are not expected before the beginning of 1987. At the press conference at the Space Exploration Institute of the Soviet Academy of Sciences last week, speakers stressed that even the initial data "leave no doubt" of the "powerful circulation" in the venusian atmosphere.

This press conference and the approach of the Soviet media in general to this, the first major space event of the Gorbachev era, is instructive. TASS reported the mission without its well-worn formula that this research was carried out in accordance with the directives of the 22nd Party Congress on the application of space to the national economy, which suggests a deliberate effort to take the public into the scientists' confidence.

One radio transmission at 16.30 Moscow time on 15 June took the form of a report from the flight control centre, suggesting that it was happening in real time. An interview with Dr Valerii Barsukov,

director of the Vernadskii Institute of Geochemistry and Analytical Chemistry, on volcanism on Venus was "interrupted" by an announcement that Veba-2 had landed. The effect may have been spoiled for discerning listeners by the fact that shorter and less dramatic announcements of the Veba-2 landing earlier that day had also been given out on radio in real time style.



The Veba-2 surface probe.

For the rest, the official spokesmen have stressed the international aspects of the mission. France has participated in providing the apparatus for the aerostatic probes and landing craft, while the Halley's comet part of the mission will involve equipment from "a large number of both socialist and capitalist countries".

Vera Rich

CERN

Schopper's views on Kendrew

REACTIONS last week to the Kendrew report on the future of British participation at CERN, the European Organization for Nuclear Research (see *Nature* 20 June, p.619), at least from particle physicists in Britain and Europe, are most diplomatically described as a blend of acute scepticism and puzzlement. The scepticism is that a combination of broader international membership and a reduced budget could possibly lead to a 25 per cent reduction in the cost of CERN by the deadline set for 1991, and puzzlement that somebody with Sir John Kendrew's experience of running the European Molecular Biology Laboratory at Heidelberg could ever have thought his panel's recommendations practicable.

Professor Herwig Schopper, the director-general of CERN, while saying he was glad that the report was very positive about the science and management of CERN, emphasized the implications of the recommendations. "Increased membership is not a new idea; some international collaborations have associate members linked to only parts of their programmes, but we have only one major programme [the construction of the electron-positron collider LEP]. Wider membership is a possibility for the future".

A major hindrance to widening CERN's membership in the near future, it seems, is that countries such as Japan and Canada (mentioned by Kendrew last week as possible candidates) would not be attracted by LEP given the advanced state of its development. Looking much further ahead, however, the proposed construction of the Large Hadron Collider in the LEP tunnel would necessitate a significant increase in CERN's budget, and that project could attract a wider membership.

The proposed cut in the cost of CERN of 25 per cent by 1991 would, in the circumstances, have to come from reduced contributions from the member states. Schopper points out that about half of the budget goes on salaries. "CERN's council could decide on lower salaries for new arrivals, but current recruitment rates are low. A decision to reduce existing salaries could result in our being taken to court." So, Schopper says, a 25 per cent cut in CERN's budget would have to come almost entirely from the materials budget, and would effectively emasculate CERN. There is no sign, moreover, that other member states would approve a significant budget cut. "This seems to be a special British problem", said Schopper.

Philip Campbell

New observatory

THE site of the new international observatory on La Palma, in the Canary Islands, which is to be inaugurated by King Juan Carlos of Spain on 29 June. From left to right the partially constructed dome of the 4.2-metre William Herschel telescope, the

IMAGE
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Swedish Solar Tower, the 2.5-metre Isaac Newton telescope, and the 1-metre Jacobus Kapteyn telescopes. Also at the ceremony will be six other heads of state (or their representatives). The participating nations are Spain, the United Kingdom, Denmark, Sweden, the Netherlands and the Irish Republic. (Photograph courtesy of the Science and Engineering Research Council.) □

British geophysics

Royal Society backs protest

THE management and financial support for geophysics research in Britain are inadequate, while government support is "poorly coordinated, even capricious". This is the theme of the report of a working party on geophysics research set up by the Royal Society under Professor E.R. Oxburgh (Cambridge). The group's report, *Support of Geophysics in the United Kingdom*, published last week, has been endorsed by the Royal Society council.

The starting-point for the working party's argument is what it sees as a mismatch between the rapid growth of reflection seismology surveys for economic prospecting, on which \$3,000 million was spent worldwide in 1982, and the scale on which basic geophysics is supported. "If there is one message we wish to convey, it is that geophysics matters to the country", said Oxburgh last week.

The report gives a gloomy account of lost opportunities in British geophysics. The next phase of the Deep-Sea Drilling Programme will "go ahead without British participation" for lack of £2.5 million a year. Except on a "private basis", British scientists will not be involved in the use of the worldwide seismic networks being built by the United States and France for modelling the inner structure of the Earth. It would be "unthinkable . . . at present" to propose British participation in the deep continental drilling programmes being mounted by West Germany or the deep-sea submersible projects of the United States and France. Noting that British geophysicists have in the past done well in elections to the fellowship of the American Geophysical Union, the working party says that the figures also show that "significant numbers of geophysicists have chosen not to work in Britain".

In its analysis of what has gone wrong, the working party says that an estimated 110 academic geophysicists and perhaps as many postdoctoral workers are distributed "in small groups or as isolated individuals" in university departments of physics, geology and even applied mathematics. The report asks for a "greater concentration in fewer centres", offering as models the Mullard Space Science Laboratory of University College, London, and the Robert Hooke Institute for Atmospheric Research at Oxford.

The mechanism of government support for geophysics research is the other chief cause of trouble. The Oxburgh group suspects that the British geophysics community may be too small a constituency of the Science and Engineering Research Council (SERC), which spent £1.2 million on geophysics research grants in 1983-84. This is nearly three times the value of the grants awarded by the Natural Environment Research Council (NERC), most of whose support for geophysics goes on its

own institutes and on collaboration ventures.

The working party is stinging in its criticism of NERC (see below), although sympathetic about the council's difficulties. The chief of these is the erosion of commissions for research at NERC institutes from government departments, which is reckoned to have declined from £25 million to £15 million (at present prices) in three years. NERC has suffered "not only its own budget cuts . . . but also

NERC pilloried

THE Oxburgh group's criticisms of NERC include the following.

● "The council, or some of its officers, sees itself as now directing research programmes rather than facilitating research in institutes and universities."

● The cost of NERC's central scientific services (mainly a computing network) seems to be too high (at £6 million a year) compared with that on other facilities and decisions about NERC services "are still being made without being approved by appropriate scientific committees".

● On the allocation of research vessel time to university scientists, the Oxburgh group complains that people cannot plan ahead. Shortly before the new research ship *Charles Darwin* was delivered last year, NERC laid up most of its other research vessels, forcing academics to put off planned observations. Because allocation of ship-time and running costs are made by different committees, investigators may be blessed with ship-time "but not the much smaller sums" required to make use of it.

● The group is concerned about the functioning of NERC headquarters. "We are astonished" at their involvement in minor matters . . . "Directors are not being allowed to direct." Apart from the unregulated growth of the computer network, the group is concerned that active scientists on the council and its committees are not "effectively involved" in the disposition of financial resources. Not surprisingly, the group raises the question whether NERC should continue to be responsible for supporting university research, says that the style of headquarters management makes NERC seem "bureaucratic and insensitive" and asks, as one of its principal recommendations, that the Advisory Board for the Research Councils should consider what should be done.

In a statement put out earlier this week, Mr Hugh Fish, now confirmed as chairman of NERC, said that he took "great exception" to parts of the report (but a spokesman declined to say what these are). Mr Fish said he plans "to do no more" than seek a discussion of the report with the president of the Royal Society. □

enhanced cuts in its contractual income from customer departments".

One result, the report says, is that NERC has been "obliged" to concentrate on work that brings in outside income "with less regard to the scientific content" and often in competition with others. The working party also says that the ability of the institutes to "compete in the marketplace" has been constrained by labour legislation and trades union agreements, citing the way in which the British Geological Survey (the largest institute) has been compelled to take onto its permanent staff a number of people originally hired for short-term projects.

In general, the report says, NERC support for its geophysics institutes ranges from the "marginally satisfactory . . . to the derisory". The Oxburgh group is especially concerned about the danger that geophysical survey work is being neglected. It says that geophysical surveys in the South Atlantic were drawn on by the British government during the Falklands war, contributing to an unquantifiable "saving in lives". The report goes on to say that "strategic national interests are involved, and it is not right that such decisions should depend on the funding priorities of a particular government department at a particular moment".

Noting that the funds made available for new research projects in geophysics have declined by half over the past five years, the working party says that one consequence has been "a pervasive pessimism and loss of morale" among researchers.

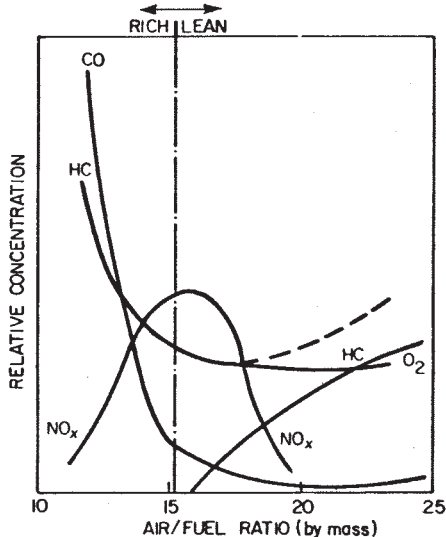
The working party is also critical of the lack of coordination between research councils and government departments. Projects falling between NERC and SERC face a "double hazard", where each council has to commit funds before work can go ahead. The report cites the "discouraging history" of a satellite laser-ranger suggested by the Royal Greenwich Observatory, where one council was prepared to meet the high capital cost but the other unable to afford the running costs.

In resignation, the working party turns from British orthodox sources of funds to those that might be found elsewhere. On the basis that much future geophysics will hang on satellite observations, the Oxburgh group asks for greater involvement by the British Department of Trade and Industry, and urges the setting-up of a space research directorate (which has since been agreed). It asks that there should be closer coordination with the military on geophysical problems of mutual interest, and says that British geophysics should take advantage of the interest of the European Commission in the geophysics of energy problems. The report says that industrial companies should spend more on the support of British geophysics, and that problems of commercial secrecy are by the Norwegian experience not the impediments to collaboration they are often said to be. □

Pollution from automobiles

SIR — A recent article by A. Lubinska¹ discusses the relative merits of catalytic converters versus lean-burn engines. In that comparison she states that "The high air/fuel ratio of the lean-burn engine lowers both carbon monoxide and nitrous oxide emissions, but increases hydrocarbon emissions...."

Nitrous oxide, N_2O , is emitted only in small quantities by internal combustion engines, while nitric oxide, NO , is produced in large quantities². In addition, N_2O is inert in the lower 10 km of the atmosphere while NO plays a major role in both photochemical smog (O_3) forma-



Variation in combustion products as a function of air/fuel ratio^{4,5}. NO_x is the sum of NO and NO_2 ; these two compounds are rapidly interconverted in the atmosphere. The dashed line shows the result of misfiring.

tion and acid deposition. She appears to have confused the two gases.

More importantly the article tends to oversimplify a complicated issue. Lean-burn engines have advanced significantly in recent years, but they are technically complex and not immediately available for mass-production. Not all types of vehicles are suitable for lean-burn engines; the increase in gasoline (motor fuel) economy is accompanied by a decrease in power.

As the air/fuel mixture becomes leaner (higher on figure), NO emissions increase before they decrease. Lean-burn engines must run at an air/fuel ratio of 22 to 25 before nitric oxide emissions are significantly reduced. At such lean mixtures, ordinary engines misfire and hydrocarbons rise dramatically. A lean-burn engine must have an efficient fuel-injection system to avoid misfiring⁶. The more advanced fuel injection system and other modifications add to the price of an automobile, expenses neglected in Lubinska's article.

Catalytic converters are more readily available than lean-burn engines but even the implementation of catalysts in European cars is non-trivial. They have been

used successfully in the United States but Europe offers more extreme driving conditions. There are more congested cities where the catalyst tends to be too cold and autobahns where routine speeds approach 160 km per hour (100 miles per hour) and the catalyst tends to be too hot.

Lead, even in very small amounts, poisons catalytic converters. In the United States, unleaded gasoline is federally mandated and nearly all driving is within national boundaries. In Europe, where drivers frequently cross national borders, lead-free gasoline may not be uniformly available.

In summary, lean-burn engines are a promising alternative to catalytic converters, but they too have problems. The reduction of air pollution from automobiles is not simple, and a variety of techniques is sure to be needed.

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Research councils

SIR — Whatever the faults of Whitehall, it is never lost for ideas to make research councils more efficient. Last year the Comptroller and Auditor General¹ warned that by appointing the best candidate for the job, the research councils were being flooded with staff of unnecessarily high calibre. This was part of the wider problem of fluid grading. Several research councils, although not the Medical Research Council (MRC), were guilty of basing promotion on the ability of the individual, rather than the name of the job. These suggestions were made in all seriousness and the research councils have duly promised to mend their ways.

It has now been realized that what research councils need, to make them more effective, is flexibility through more short-term and fewer career appointments². It is well known that a civil servant who shows signs of starting to understand a job must be moved on as quickly as possible. This strategy, which has done so much for the British civil service, could well be expected to transform the work of the research councils.

There are one or two difficulties. The Advisory Board for the Research Councils has itself pointed out² that scientists on short-term contracts tend to spend the first half of their time learning to do the work and the second half looking for

another job. Moreover, the absence of any prospect of employment after the age of 35 could conceivably deter first class candidates from applying. The research councils hope that with some alteration of pension arrangements those on short-term appointments will move on to jobs in industry though, outside a few narrow specialties, it is far from certain that this is what industry wants.

PhDs with postdoctoral experience are deemed overqualified for the majority of jobs in industry. Most of the remaining jobs require industrial rather than research council experience. As an indication, over the past 3 years, 230 MRC scientists came to the end of short-term appointments; 13 obtained permanent appointments with MRC and 14 obtained jobs in industry. Most of the remainder went on to yet another short-term appointment. It would be no harm if MRC were permitted to let short-term staff benefit from the pension contributions made on their behalf, rather than to use these contributions, as at present, to subsidize the pensions of other staff members, but it is doubtful if this would fully compensate for such appalling career prospects.

Scientists vote with their feet, and short-term appointments tend to produce few applicants, often of indifferent ability. Permanent appointments attract large numbers of applicants, many of outstanding ability. It has never been explained why it is thought that employing less able scientists on disadvantageous terms will produce greater flexibility and effectiveness than employing more able scientists on advantageous terms. Perhaps the only moral is that the research councils are capable of mismanaging their affairs quite badly enough without the assistance of Whitehall.

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Embryo research

SIR — In the controversy over the status of the human embryo, there exists a state of doubt: some say it is a human being, others say it is not. The views seem fairly equally balanced.

In this impasse, the next step is to examine the consequences should one side or the other eventually be proved to be indisputably correct. On the one side we risk losing a valuable research opportunity — on the other we risk committing murder.

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Observational plate tectonics

The latest report of the international radio-interferometric survey IRIS suggests that the direct measurement of plate movements lies just ahead.

THE exciting prospect that radioastronomers will be able, by the use of long-baseline interferometers, directly to measure the motion of tectonic plates, will be a little delayed, it seems. That is one cautious reading of the latest report on four years of work with the international network of geodetic observing stations operated under the title IRIS (for International Radio Interferometric Surveying), which is itself an outgrowth of the combined operation of the US project POLARIS (for Polar Motion Analysis by Radio Interferometric Surveying) and the West German geodetic radio telescope at Wettzell, Bavaria. In an account of observations to the end of November 1984, W.E. Carter, D.S. Robertson and J.R. MacKay of the US National Geodetic Survey (*J. geophys. Res.* **90**, 4577–4587; 1985) quote estimates of the rate at which two baselines are changing, but with errors that are still too great for comfort.

The idea that radio-interferometry might be used for checking on plate tectonics has been in the air ever since radio telescopes separated by a substantial distance were first used for the accurate measurement of the angular size of distant radio sources, quasars for example. But the technology of very long baseline interferometry (VLBI) is only now good enough, and the network of observatories enough, to bear on plate tectonics.

Schematically, the technique is simple enough. Several telescopes simultaneously observe the same distant radio source, recording their signals on magnetic tape (together with an accurate measure of time, most conveniently provided by a local hydrogen maser). In general, there is a time-lag between the receipt of the same waveform at one station and another which, when multiplied by the velocity of light, will be a simple measure of the separation of the two antennas along the line of sight. But given that the Earth rotates, the time-lag will change systematically with time, and in such a way that it should be possible to infer from the observations not merely the relative geometrical position of the two antennas, their baseline separation included, but also the instantaneous position of the axis about which the Earth is rotating.

Practice is, as always, more difficult. One heartfelt complaint by Carter *et al.* is that the sheer weight of the magnetic tape which has to be assembled at a comparator centre after a day's observation by a hand-

ful of stations may amount to several hundred kilograms, "a troublesome constraint limiting the growth of geodetic VLBI". Even so, the planners intend adding to the four core stations (Westford, Massachusetts, the Agassiz station in Texas, Richmond, Florida, and Wettzell) cooperation with the Kashima Observatory in Japan and the Shanghai Observatory on mainland China. Other radio telescopes in the United States and elsewhere join in the network as the opportunity arises; that at Onsala, Sweden, takes part for one day a month.

The most startling result so far seems to be that of the measurement of the Earth's pole position, which has been determined since the beginning of 1984 with an accuracy of 2 milliarc seconds (or 6 cm on the ground). This, at least, is the discrepancy between the VLBI measurements of the pole position and those obtained quite independently from satellite ranging, which confirms the accuracy of both sets of measurements. VLBI measurements every three or five days may be enough to keep track of this polar (Chandler) wobble to within a milliarc second.

For what it is worth, the 1984 observations put the position of the pole on a smooth arc destined to form a near-circle some 16.5 metres in diameter, not very different in amplitude from that measured in the previous year but more than twice as great as the amplitude of the Chandler wobble in the two earlier years. Because the VLBI measurements will be made in any case, and because they can be quickly reduced, they will quickly become the preferred way of tracking the wobble.

The data have also thrown up a wealth of detail about departures of the Earth's rotational speed as given by Universal Time (strictly, UT1). Departures from regularity leading to fluctuations of up to 6 milliseconds occur, with some excursions lasting for several months and others for only a few days. It should be possible to monitor the regularity of UT (or of the Earth's rotational speed) to within 0.1 milliseconds by means of a daily hour-long measurement along a single baseline. That is also a by-product well worth having. Daily monitoring may even make it possible to tell which fluctuations of rotational speed are attributable to what.

Other by-products include some information on the nutation of the Earth's rotation, which reflects displacements between the spinning core of the Earth and

the outer solid hollow sphere. The Earth tides responsible for the rhythmic fluctuation of the altitude of the radio telescopes in the network are also evident in the data.

So what is there to be said about the baselines and their variation? It is obviously easier to extract from the measurements of the time-lags and their variation with time the quantities whose time-dependence takes a predictable form. This is one reason why fluctuations of the Earth's rotational speed can be monitored so easily; the time-lag between any pair of stations in the VLBI network will be a sinusoidal function of time whose frequency is that of the Earth's rotation.

By contrast, the baseline lengths appear as factors in the constants by which these variable quantities are multiplied, so that the accuracy with which they can be determined is no greater than that of the observations as a whole. Even so, baseline measurements are said to be repeatable to within about one part in 100 million, a mere centimetre or so of error for the longest baselines, for the time being those based in Europe and the United States.

This is the promise for plate tectonics. Many of the movements expected should be readily verified as further measurements of the baselines accumulate. Indeed, Carter *et al.* tantalize their readers with an estimate of the rate of change of the baseline between Westford (Massachusetts) and Onsala (Sweden) and that between Westford and the Texas station. The conclusion is that the trans-atlantic baseline is lengthening by 14 mm a year (with an estimated uncertainty of 3 mm) and that the baseline between Westford and the Texas station is shrinking by 8 mm a year (with an uncertainty of 1 mm).

The sense of these results, at least, is consistent with what plate tectonics would predict; the contraction of the US baseline, for example, is thought to be a consequence of compressional stress on the American plate at least in the region between the Appalachians and the Rockies (see D. I. Gough *et al.* *Nature* **305**, 619–621; 1983). The fly in the ointment is the suggestion of seasonal errors in the baseline measurements, implying a need for some refinement of the corrections for transmission through the ionosphere and atmosphere. The general interest now will be to see how quickly the VLBI collaboration will be able to accumulate further data. Another year's observations should make all the difference. **John Maddox**

Meteorites

Earthward bound from chaotic regions of the asteroid belt

from Carl D. Murray

It is easy to accept that meteorites, those occasional stones that fall from the sky, are composed of asteroidal material that has been delivered to the Earth. The problem has always been to demonstrate how, subject to some severe observational constraints, even a single fragment in an asteroid-type orbit can eventually cross the path of the Earth. On page 731 of this issue, Jack Wisdom of the Massachusetts Institute of Technology, guided by known locations of chaotic regions in the phase space at one particular place in the asteroid belt, describes a trajectory that can evolve from a fairly typical low-eccentricity orbit to the high eccentricity necessary to achieve the elusive Earth-crossing orbit.

While there are suggestions of a lunar or even martian origin for some of the meteoritic material that has been examined, photometric analysis of most meteorites strongly supports an asteroidal origin. Meteorites are typically thought to be fragments arising from a minor collision between two asteroids. (The asteroid belt is continually evolving because of collisions, which have average encounter velocities of 5 km s^{-1}). The fragments, which will have slightly different orbital parameters from their parent bodies, eventually enter Earth-crossing orbits.

Although the details of this final process are not known, we have some idea of how long it takes. Any fragment that was initially buried more than a few metres inside its parent body will have been shielded from the effects of cosmic rays up to the moment of impact. From chemical studies of meteoritic samples, it has been possible to calculate for how long they have been exposed to cosmic rays and so when the collision which generated them occurred. The exposure ages are generally around 10^6 years for the common stony meteorites, putting severe time constraints on any delivery mechanism.

Regions of the Solar System that might be expected to act as a source for meteorites fall near the low-order orbital resonances of Jupiter. An asteroid occupying the 2/1 resonance, for example, orbits the sun in half the orbital period of Jupiter. Thus, over successive orbits the asteroid will experience a repeated and cumulative gravitational perturbation from Jupiter that could tend to remove it to a different orbit. Prominent gaps (Kirkwood gaps) in the asteroid belt occur at such resonances and Wisdom has already demonstrated¹ how the 3/1 resonance can be cleared of asteroids. He showed the existence of a large chaotic region of the phase space

(defined by positions and velocities) in the vicinity of the resonance. In such a chaotic region an asteroid could experience large and essentially random changes in its orbital parameters, with the final orbit being sensitively dependent on the initial conditions. The key point is that the equations of motion are non-linear and the resulting deterministic chaos is now being recognized as a common feature of non-linear equations for certain sets of starting conditions. The most critical orbital parameter for the asteroid's survival is its eccentricity. A value larger than about 0.3 implies that it will cross Mars. Having shown how such eccentricities could be achieved in the chaotic zone, Wisdom invoked Mars as the final remover of material, whether by impact or scattering. It seemed natural, therefore, to use knowledge of the locations of the chaotic regions as a starting point in a search for regions that could produce Earth-crossing material.

In a paper presented to the Meteoritical Society last summer, George Wetherill of the Carnegie Institution in Washington DC, claimed that the observed influx of the ordinary chondrite meteorites could be accounted for only by gravitationally accelerated material from a region in the vicinity of the 3/1 resonance³. Wisdom has now provided details of such a mechanism using the known locations of the chaotic regions as a basis for finding Earth-crossing trajectories. When he integrated

different sets of starting conditions, at the fifth attempt he found a trajectory which became Earth-crossing. The equations he integrated have sufficient degrees of freedom for Arnol'd diffusion to occur — whereby an orbit, given enough time, can eventually 'leak' into different regions of the phase space². (Arnol'd diffusion has yet to be identified in any Solar System integration since the timescales involved are almost certainly greater than the age of the Solar System.) Wisdom's result is that the orbit evolves from low to high eccentricity within a few hundred thousand years, thereby satisfying the cosmic-ray exposure age constraint. The beauty of the mechanism is that it does not involve any complicated procedures that require more than one close approach to another planet.

The 3/1 resonance does not have a monopoly on chaotic regions — the boundaries of all resonances are chaotic, the extent of the chaos depending of such factors as Jupiter's eccentricity and the strength of the resonance. In the past, celestial mechanicians have devoted much effort to the study of the prominent first-order resonances in the asteroid belt, trying to explain such features as the 2/1 Hecuba gap and the 3/2 Hilda group of asteroids. It now seems that an understanding of the dynamics of the innocuous 3/1 resonance will provide answers to some of the most fundamental problems of Solar System dynamics. □

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Avian ecology

Mass mortality in Pacific seabirds

from Jared M. Diamond

WHAT is the evolutionary importance of rare events? Does natural selection proceed constantly, or are its effects concentrated during episodes of widespread mortality? R. and E. A. Schreiber (*Science* **225**, 713; 1984) address this question for some of the world's longest-lived vertebrates, victims of a recent catastrophe.

El Niño Southern Oscillations (ENSOs) are recurrent, widespread climatic events involving major warming of the whole eastern tropical Pacific Ocean. The most recent ENSO, in 1982–83, was the most severe yet recorded. At the site of the Schreibers' study — the low atoll of Christmas Island in the central Pacific — annual rainfall, normally 30 inches, reached 156 inches in the year beginning July 1982, including 16 inches in one day.

Among the consequences were extensive flooding of the island, a drop in oceanic primary productivity of phytoplankton dependent on cold surface waters, and hence depleted stocks of fish and squid ultimately dependent on that primary production. Eight million seabirds of the eighteen species that breed on Christmas Island feed on those fish and squid. Although most temperate zone song-birds breed in their second year, lay 3–10 eggs and have life expectancies of a few years, tropical seabirds typically have a single large egg with a long incubation time, long periods of juvenile dependency on parents, late onset of breeding and very long lives. Long-term studies of Christmas Island seabirds have revealed many details of their leisurely life cycles. Some

species have lifespans of 50 – 70 years, more than the lifetime of the rings used to tag them. The tropic birds breed only after the age of 5 years, live at least 35 years and form permanent pairs. A young frigate bird is fed for about one and a half years by its mother (the father leaves after 1 month, probably to nest with another female). Fledged sooty terns fly to sea, do not alight for 9 years (presumably sleeping in flight), then infallibly return to their natal island, where they court on the wing, finally alight to copulate, and lay the egg on the next day.

From 1940 to June 1982, a period that included seven ENSOs, there was no recorded instance of total reproductive failure for any seabird species at Christmas Island. During the Schreibers' June 1982 visit, just before the onset of the ENSO, seabird populations were still normal. But by November 1982 there was total reproductive failure for all species. At that time five species (including the two that are usually most abundant) were completely absent, while the remaining species were represented by few adults, with no nests or else nests with dead, underweight or starving chicks. Nests in ground burrows were flooded, many tree nests had been washed out and young in surviving nests starved.

What happened to the millions of adult birds that should have been breeding then on Christmas Island? Did they, too, die, or did they simply go elsewhere until conditions improved? The Schreibers found almost no skeletons of adults on the island, yet bird numbers were still far below normal in October 1983. It is thought that 90 per cent of the breeding seabirds, including those present and attempting to breed in July 1982, died at sea from starvation.

The low reproductive potential of tropical seabirds suggests that it will be years before the breeding colonies of Christmas

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Frigate bird fishing — one species of seabird that breeds on Christmas Island (photograph courtesy of R. and E. A. Schreiber).

Island have recovered to their former numbers. Thus, a large-scale natural experiment in fluctuating selection is underway. Intraspecific competition will be relaxed for a long time: how will this affect reproductive effort and individual variation in morphology? Was the mortality in 1982 indiscriminate and total, as presently assumed, or did a few of the fittest survive? These long-lived birds must experience many 'normal' ENSOs in their lifetimes, and a severe one every couple of generations; could there be features of the life histories of the birds that have arisen as adaptations to these rare events? The answers to these questions may emerge from the long-term studies on Christmas Island. □

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Meteorology

Clouds and climate regulation

from Richard C. J. Somerville

THE role of clouds in regulating climate is still largely a mystery, and the treatment of clouds in conventional climate models may be seriously inadequate. If cloud effects are as strong as some recent research suggests, considerable uncertainty must characterize the results of even the most advanced present-day models of climate. Predictions of the climate changes resulting from burning fossil fuels, which adds CO₂ to the atmosphere, for example, depend strongly on how clouds are modelled; clouds may tend to amplify changes, or they may act as a giant global thermostat, helping to stabilize the climate.

The potentially large influence of clouds on climate arises from their effects on the transfer through the atmosphere of

both incoming solar and outgoing terrestrial radiation. That clouds may dramatically affect surface temperature by both these mechanisms is a matter of everyday experience. Daytime maximum temperatures are substantially lower on cloudy days than on fair ones, and cloudless nights are cooler than overcast ones, other factors being equal. These commonplace examples illustrate the two major influences of clouds on the planetary radiation budget: clouds can alter both the planetary albedo, or reflectivity, and the greenhouse effect, by helping to trap radiation which otherwise would be lost into space.

The average albedo of our planet is about 30 per cent, so only 70 per cent of

incident solar radiation is actually available to power the climate system. Clouds cover nearly half the surface of the Earth but account for about two-thirds of the planetary albedo¹. We do not know whether the albedo or cloud cover were markedly different during, say, the last ice age. In fact, we have no good explanation of the present-day values of these fundamental climatic parameters. It is perhaps even more disquieting that we cannot reliably predict whether or how these parameters are likely to change over the next century, as the concentration of atmospheric CO₂ approaches twice its pre-industrial value. It has recently been estimated, for example, that the temperature increase due to the greenhouse effect produced by doubling CO₂ might be completely offset if the amount of low-altitude cloudiness were to increase simultaneously by 10 per cent². This result emerges from a mathematical model sensitivity study, essentially a calculation of the climatic response to an assumed change in cloudiness.

The predicament of climate theorists is that it is relatively easy to demonstrate that climate is potentially sensitive to clouds — as in the example above — but very difficult to model clouds realistically. Climate models incorporate powerful radiative transfer algorithms that could calculate atmospheric heating and cooling extremely accurately, if only the distribution of all the radiatively active constituents in the atmosphere were known precisely³. Two of the most important such constituents, however, are water vapour and cloud, both notoriously changeable and difficult to predict. Idealized mathematical models have been developed to simulate many of the physical mechanisms through which a change in climate might alter clouds, which can then in turn feed back on climate change through their radiative effects^{4,5}. Not even the most advanced of climate models, however, simulate all aspects of the present-day global distribution of cloudiness well enough to inspire confidence in cloud forecasts for climates differing significantly from our present one⁶.

Many sensitivity studies have shown that climate can be affected strongly by changes in cloud height as well as changes in cloud amount⁷. Recent research has raised the additional possibility that increased atmospheric pollution might alter cloud optical properties enough to influence the global albedo substantially⁷. Another mechanism which might produce a similar effect is the possible dependence of cloud liquid water content on temperature⁸. In the context of the CO₂ climate problem, both these processes are candidate cooling mechanisms that might at least partially compensate for the warming resulting from the enhanced greenhouse effect. The quantitative importance of these processes is still uncertain, but they illustrate the real possibility that

the effect on climate of feedback from cloud radiation may be significant even in the absence of changes in cloud amount or height.

One important reason for the frustratingly slow progress in this field has been the lack of adequate global cloud observations. Satellite remote sensing has only recently begun to fulfill its considerable promise in this area⁹⁻¹¹. Parameters that can now be measured with useful precision include cloud amounts, cloud heights, cloud-top temperatures and some optical properties of clouds. Earth radiation budget parameters, such as albedo and outgoing longwave radiation, are now also being monitored by satellites. Although no dramatic breakthroughs seem likely, these data will be invaluable in the gradual development and verification of improved climate models.

For those who are unwilling to wait a century or so to learn whether CO₂ doubling will or will not have its predicted climatic consequences, there may be a quicker payoff from research on cloud-radiation feedbacks. Numerical weather prediction models, on which routine operational meteorological forecasts are based, have become so complex that they are now almost indistinguishable from comprehensive climate models, but the conventional wisdom has long been that short-range atmospheric circulation forecasts, for a day or so in advance, are relatively insensitive to radiative processes. Indeed, such processes were until recently highly simplified or even entirely neglected in the forecast models used by national weather services. As the duration of the predictions increases, however, such cavalier physical simplifications become less justifiable. There is now considerable evidence that a one-week forecast may benefit significantly from more realistic

treatments of cloud-radiation interaction^{12,13}, and improvements in modelling clouds and their effects are likely to increase gradually the competence of an already skilful enterprise. If research on modelling cloud effects can lead to real improvements in one-week forecasts, the benefits to many weather-sensitive sectors of the economy should be substantial.

It is climate theory rather than numerical weather prediction, however, that stands to gain the most from any real advances in our understanding of cloud-radiation feedbacks. It is already clear from sensitivity studies that cloud feedbacks could plausibly either double or halve the typical estimated surface temperature change resulting from increased concentrations of atmospheric CO₂. But our present ignorance of these feedbacks is so great that we cannot even be certain whether the net effect of all the cloud processes is a positive or a negative feedback. □

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Developmental neurobiology

Laminin for axonal guidance?

from Joshua R. Sanes

INTERACTION of neuronal processes with the surfaces on which they grow has been a subject of increasing interest to developmental neurobiologists. *In vitro*, it is clear that adhesion of growing processes, or neurites, to substrates is an important determinant of neurite elongation¹ and that material deposited by non-neural cells can promote and direct neurite extension². *In vivo*, regenerating peripheral axons frequently grow along basal laminae to reach and reinnervate their targets³. These two observations have now been brought together in a satisfying way by the demonstration that laminin^{4,5}, a structural component of adult basal laminae⁶, is a major factor promoting neurite outgrowth that is secreted by cultured cells.

In 1978, Collins² demonstrated that proteins secreted by heart cells promote neurite outgrowth in a way that distinguishes them from soluble 'trophic' factors such as nerve growth factor (NGF): they bind to the culture dish and seem to act by increasing the adhesion of neurite to substrate. Many cell types have since been shown to secrete such material⁷ and efforts are now being made to characterize the responsible factor. These have proceeded along two lines. First, the outgrowth-promoting activities have been purified from medium conditioned by non-neural cells, using neurite outgrowth as an assay. Second, based on the finding that many proteins secreted *in vitro* are components of extracellular matrices *in vivo*, the effects of purified extracellular matrix

molecules on neurite outgrowth have been assessed and compared to those of outgrowth-promoting factors. Although several such molecules turn out to be effective substrates for neurite outgrowth, most attention has focussed on laminin, a glycoprotein that is present in virtually all basal laminae and that had already been shown to promote attachment of epithelial cells to collagenous substrates⁸. When tested for neurite outgrowth-promoting activity, laminin seemed to be the most effective of the molecules examined, to affect the widest variety of neuronal types and to be remarkably similar in its effects to the outgrowth-promoting factors secreted by cultured cells⁸⁻¹³.

As these results accumulated, it became logical to suppose that the secreted outgrowth-promoting activity might actually be laminin, but two observations suggested that it was not. First, antisera to laminin that blocked neurite outgrowth on laminin-coated dishes did not inhibit outgrowth on substrates coated with conditioned medium^{4,9,11}. Second, Lander *et al.* found that the factor from conditioned medium contains a heparan sulphate proteoglycan, although they appreciated that the proteoglycan was not necessarily the molecule that the axons recognized¹⁴. The parallels between laminin and this factor were, however, sufficiently striking to be pursued, and now Lander *et al.*⁴ and Davis *et al.*⁵ have obtained new evidence indicating that laminin is indeed responsible for the outgrowth-promoting activity in a variety of conditioned media. Laminin co-purifies with the outgrowth-promoting activity through several fractionation steps; it is a major component of the purest factor obtained; anti-serum to laminin precipitates outgrowth-promoting activity from conditioned medium; and sufficient laminin is present in conditioned medium to account for outgrowth-promoting activity. The laminin is likely to be complexed with the heparan sulphate proteoglycan previously described¹⁴, because anti-laminin precipitates both molecules from medium. Furthermore, while heparan sulphate proteoglycan alone does not stimulate neurite outgrowth⁹, a monoclonal antibody that blocks the outgrowth-promoting activity of conditioned medium¹⁵ recognizes a complex of laminin with proteoglycan (W. Matthew, personal communication).

The failure of anti-laminin to block the activity of the complex remains unexplained and provocative. The laminin molecules in the complex may differ in their primary structure, glycosylation or conformation from the laminin used to generate the serum, or the active site of the complexed laminin might be inaccessible to antibody. It seems odd that the active site of the laminin could be accessible to an axon but inaccessible to an antibody; one interesting possibility is that heparan sulphate on a neuronal mem-

brane could displace heparan sulphate in the complex, giving the axon special access to an active site. In any event, these results argue for caution in interpreting experiments designed to identify active molecules by immunological blockade.

The findings that laminin promotes neurite outgrowth and is a major component of the outgrowth-promoting factor secreted by several types of cells have several implications for research on axonal growth and guidance. First, neurites can be predicted to bear laminin-receptors⁶ on their membranes, so it becomes important to identify and characterize these molecules. Second, we will want to learn if and where laminin guides neurite outgrowth *in vivo*. Although the immunochemical difficulties I have already discussed may render interpretation of antibody-blocking experiments difficult, it should be possible to learn how far outgrowth on basal laminae depends on laminin as opposed to other extracellular matrix molecules such as fibronectin and collagen, which also promote neurite outgrowth *in vitro*^{8,10,16,17}. Since in the embryo central axons grow along basal laminae to reach their targets¹⁸, guidance by laminin may be relevant to the growth of central as well as peripheral axons. Finally, the clinically disastrous failure of mammalian central axons to regenerate following injury is now suspected to be caused, at least in part, by a deficiency in their environment,

because they can grow long distances through grafts of peripheral nerve segments rich in basal lamina¹⁹. Intriguingly, fish optic nerves, which have an unusual ability to regenerate, have very recently been found to accumulate high levels of laminin after injury¹³. Could laminin be one of the factors whose absence impedes regeneration in our brains and spinal cords? □

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Astrophysics

Optical bursts – where are they?

from K. Hurley

DESPITE their brevity, and sometimes because of it, transient events are often the most spectacular in astrophysics. Gamma-ray bursters¹, now widely believed to occur on or about neutron stars, are one example; typically they produce a blast of radiation lasting several seconds and with a peak luminosity of at least nine orders of magnitude over the quiescent state, in which they are quite undetectable. Transient phenomena in the optical waveband, apparently associated with some bursters^{2,3} have also been detected but, until recently, only on archival photographic plates. Therefore, they were not time-resolved. Now that continuous monitoring of optical bursts is being undertaken, the evidence indicates that they have light curves which strongly resemble their progenitor gamma bursters, and a clever argument has been advanced which uses this fact to constrain the still unknown distances to bursters.

Our present understanding of the gamma-burst phenomenon is still quite incomplete. It is much easier to list the objects with which gamma bursters are not associated, such as steady X-ray and γ -ray sources, than it is to identify convincingly

any quiescent counterpart to them. This has proven to be perhaps the major obstacle to progress, as it leaves the distance to the source uncertain by four orders of magnitude. So, although a wealth of circumstantial evidence points to strongly magnetized neutron stars as burst sources in at least some cases, the question of the type of stellar system — if any — in which the neutron star resides has still not been answered.

Perhaps nowhere has the problem been so acute as with the fascinating burster of 5 March 1979⁴: not only was its peak intensity (at Earth) the largest recorded for a gamma burst, but also its localization coincides with the N49 supernova remnant in the Large Magellanic Cloud (LMC), at a distance of 55 kiloparsecs. A straightforward interpretation would be that the burst originated on the neutron star which produced the remnant. But a number of objections can be raised to this idea, not least that, if the source radiated isotropically from the LMC, then for a brief instant it outshone (in γ -rays alone) the entire luminous output of our Galaxy. A tantalizing new piece of evidence was announced last summer at a meeting at

Stanford University and subsequently in *Nature*⁵. During a five-month period of optical monitoring of the 5 March source, three optical bursts were detected and clearly time-resolved. Their light curves generally resembled the time histories of gamma bursts, although no simultaneous γ -ray activity was noted at the times of the optical bursts (the instruments in orbit at the time were probably not sensitive enough to record it). Thus, the relation between the 5 March gamma bursts and the optical bursts remains tentative. However, as R. London of Lawrence Livermore Laboratory was quick to point out, if the two bursts are associated, the distance to the source is strongly constrained, an idea later confirmed by J.-M. Hameury, J. P. Lasota and S. Bonnazola of Meudon Observatory.

The argument is that if the optical radiation is due to a thermal mechanism (for example, reprocessing of gamma radiation⁶ in an accretion disk or in the atmosphere of a stellar companion), the luminosity of the mechanism cannot exceed that of black-body radiation. Then, since the dimension of the optical source cannot exceed ct , where c is the speed of light and t is the rise time of the optical flash, a relation is obtained between the maximum distance to the source and its temperature. That temperature turns out to be at least several million electron volts for a source in the LMC. In general, it is difficult to construct thermal models with these temperatures and furthermore, the 5 March burster has never been observed to emit at these energies. The conclusion is that its source is much nearer than the LMC. In arriving at this conclusion, a number of special models for the optical radiation have to be excluded: coherent emission, sources in relativistic expansion, beaming and special geometries that appear to violate causality.

In addition to providing distance constraints, time-resolved optical flashes may do for γ -ray bursters what they did for X-ray bursters⁷, that is, allow an estimate of the dimension of the reprocessing region (via both the rise time and the duration of the flash). In the 5 March case, for example, a reprocessing region with a typical dimension of 30,000 km is indicated, ruling out reprocessing in the atmosphere of a non-degenerate stellar companion and suggesting the possibility of an accretion disk.

But what of the tentative nature of the association between optical flashes and gamma bursters? There has never been any simultaneous observation of the two, and since optical instruments tend to be many orders of magnitude more sensitive (as energy-flux detectors) than γ -ray burst instruments, for the time being only optical flashes associated with the largest (relatively infrequent) gamma bursters can be expected to give rise to coincident detections. Simultaneous direct imaging and photometry of an optical flash should

soon be possible with a unique system now being deployed at the European Southern Observatory. The experiment, being conducted by H. Pedersen, consists of a battery of small automated telescopes each trained on the position of a particular γ -ray burst source.

Much of the uncertainty surrounding optical flashes comes from the possibility that they are generated rather closer to home, for example by meteors in the upper atmosphere. The use of two detection systems separated by even one kilometre eliminates these effects by parallax. Such a study was recently undertaken on an intercontinental baseline⁸ and another two-telescope proposal is under consideration by the European Southern Observatory. On top of this, a novel electronic camera array being installed at Kitt Peak National Observatory^{9,10}, will eventually provide permanent night-time monitoring of much of the sky for optical transients down to magnitude 11, with source localization at the arc-second level. It is expected that some twenty optical flashes associated with gamma bursts will be

detected per year (not to mention a host of other short-timescale astronomical events), dramatically increasing the probability of a simultaneous optical and gamma-ray detection. This set up will also greatly increase accuracy of localization, in the wake of which may come both the long-awaited identification of the counterpart and the answer to the question of the distance scale. $\square$

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Laser physics

Trapping cooled sodium atoms

from B. W. Petley

SPECTROSCOPISTS have long dreamed of being able to make measurements on atoms or ions which are essentially at rest, and even on single atoms in that condition. For if they could do so, the study of fine detail of atomic transitions would no longer be impeded by the broadening of spectral lines as a consequence of the Doppler effect, or by the fact that, in work with beams of atoms and ions, the accuracy of observations is limited by the time spent by individual atoms and molecules within the action region of the apparatus. One practical consequence could be the further refinement of the accuracy of atomic frequency standards.

With the recent publication of techniques for using lasers to 'cool' atoms, or to rob them of translational velocity, and with the availability of electromagnetic traps for small numbers of atoms, the dream seemed about to come true. Now, Alan L. Migdall, John V. Prodan and Bill D. Phillips of the Gaithersburg laboratories of the National Bureau of Standards, together with Thomas H. Bergeman and Harold J. Metcalf of the State University of New York, Stony Brook, have reported the trapping of laser-cooled sodium atoms. The results were presented at the spring meeting of the American Physical Society on 24 April 1985 and have just been published (*Phys. Rev. Lett.* **54**, 2596; 1985). Other groups are rumoured to be close to success as well. Thus, Metcalf (see opposite) reports that A. Ashkin and his colleagues have extended their laser trap-

ping techniques from 10 mm oil droplets to sodium atoms. We can expect a lively conference season in the months ahead.

The first-order Doppler effect, which is proportional to v/c for individual atoms and for a source in thermal equilibrium, usually results in a broadening of spectral lines by a few GHz (or about 0.1 cm^{-1}). The use of tuneable dye lasers in conjunction with atomic and ion beams has for some time made it possible to observe only those atoms that are travelling perpendicular to the line of sight, which for practical purposes removes the first-order Doppler broadening. For many purposes, however, the second-order (relativistic) effect, proportional to $(v/c)^2$, remains an impediment to accurate measurements.

The laser cooling techniques recently reported follow earlier suggestions by Letokhov and others in Moscow, by Schawlow and Hansch at Stanford and by Wineland and Dehmelt. A beam of atoms is decelerated by absorbing a counter-propagating beam of laser light. The objective is to cool such beams to milli-Kelvin (mK) temperatures. The trade-off with laser cooling is that, as atoms are slowed by absorbing N counter-propagating photons in the beam direction, they are heated in the transverse direction by the isotropically re-emitted photons as $N^{1/2}$, which may amount to 50 mK for 20,000 photons. As the atoms have an energy spread, it is convenient to express this as a 'temperature'.

Although laser cooling had already

been demonstrated by groups in Moscow, Washington and elsewhere, optical pumping effects, which deplete the proportion of the atoms in the state absorbing the counter-propagating radiation, had hindered the achievement of the desired mK temperatures. Each time, for example, that a sodium atom absorbs a 589 nm photon its velocity is slowed by about 3 cm s^{-1} , but optical pumping effects and Doppler shifts that occur after the absorption and isotropic re-emission of about a hundred photons, limit the velocity reduction to about 3 m s^{-1} , which is a long way short of the reduction by about 600 m s^{-1} required to bring the atoms to rest.

By overcoming these optical pumping effects, two groups have successfully cooled atoms to mK temperatures. Both used sodium atoms, whose 589-nm transition is so conveniently accessible with the rhodamine-6G laser dye, and both used the same transition (Fig. 1) using the indicated σ^+ line to slow the atoms. Diverging beams of atoms were cooled by a counter-propagating laser beam which converged at the same angle (3 mrad) onto the source. For this transition, the optical pumping effect results from the decay from an upper level into the $F=1$ ground state. In addition to avoiding populating such levels, the frequency of the incident radiation must also track the changing Doppler shift during the absorption of the 20,000 or so photons required to bring the atom to rest. Where the two groups differ is that one changed the frequency of the laser and the other the frequency of the atomic transition as the atoms are slowed.

W. Ertmer *et al.* (*Phys. Rev. Lett.* **54**, 996; 1985) swept the laser frequency using an acousto-electro-optic travelling wave modulator and added a further weak modulation 1,772 MHz side-band to pump any atoms which reach the $3S(F=1)$ state back up to the $3S(F=2)$ state. This produces a pulse of cooled atoms. On the other hand, J. Prodan *et al.* (*Phys. Rev. Lett.* **54**, 992; 1985) tuned the transition frequency by passing the atomic beam through the magnetic field produced by a tapered solenoid. The magnetic field and light polarization exploited the Zeeman effect to avoid the optical pumping. Deceleration was a two-stage process; the atoms were slowed to a low velocity in the solenoid and then drifted a further 0.4 m into the observation region where they

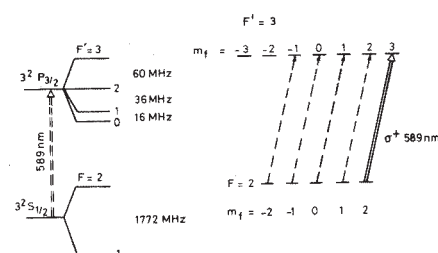


Fig. 1 Transitions involved in the laser cooling of ^{23}Na atoms by Ertmer *et al.*, Migdall *et al.* and Prodan *et al.* (not to scale).

received a further 0.1 to 0.4 ms pulse of cooling laser radiation. The method produces position-dependent cooling. With a strongly saturated transition, the atoms will spend about a half of their time in the upper energy state, radiating at a rate governed by their natural lifetime of about 16 ns. Thus their maximum deceleration would be about 10^6 m s^{-2} , which means that a sodium beam could be stopped in about 1 ms, after travelling a distance of about 0.5 m. The deceleration achieved by Prodan *et al.* was about half maximum.

The temperatures of the cooled atoms were monitored by the fluorescence produced by a weak laser beam crossing the atomic beam at a convenient angle. Ertmer *et al.* estimate that some 10^6 atoms per ml could be cooled to a velocity distribution whose width corresponds to a temperature of 50 mK (25 MHz half-width). The corresponding velocity spread obtained by Prodan *et al.* was equivalent to about 100 mK with a density of about 10^5 atoms per ml. For their subsequent successful trapping of some of their cooled atoms, Prodan and his colleagues used a trap comprised of two opposed coils

(reminiscent of an opposed Helmholtz pair); the downstream coil is energized, once the atoms have drifted into the trap. The coils produce a field gradient of about 1 T m^{-1} , which reflects and traps atoms with an axial temperature of below 17 mK. The upstream coil provides a gradient field which is used in the final stage of laser coding once the atoms have entered the trapping region. The atoms then spread out to fill the 20 ml volume of the trap and some 10^4 atoms are trapped for about 0.83 s (the decay time). The next stage will be to attempt to use optical trappings to cool the atoms even further so that they are sufficiently at rest to dispense with the trap. Meantime many exciting experiments are in prospect.

Atomic frequency standards

To appreciate the possibilities of the new technique one has only to consider a paper of J. J. Bollinger *et al.* (*Phys. Rev. Lett.* **54**, 1000; 1985) in which the successful operation of an atomic frequency standard based on laser cooled ions is reported. Bollinger *et al.* trapped $^9\text{Be}^+$ ions in a Penning trap, whereby the ions were con-

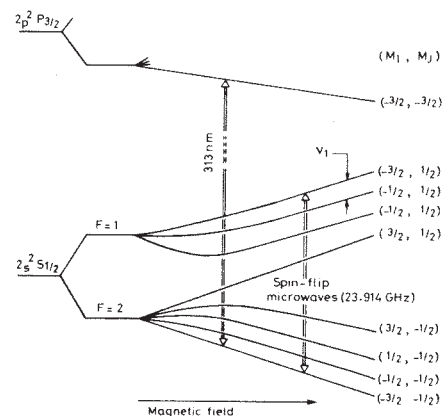


Fig. 2 The Zeeman splitting in a magnetic field, and the hyperfine structure of $^9\text{Be}^+$ showing the transition used for laser cooling by Bollinger *et al.* (not to scale).

Optically trapped oil droplets

IN A recent article in *Physical Review Letters*¹, A. Ashkin and J. Dziedzic of AT&T Bell Laboratories report the first stable trapping and manipulation of single minuscule oil droplets using only the forces derived from the pressure of laser light. Although the force exerted by light on any object is normally so tiny that it is not of importance, the special circumstances contrived by the experimenters make it the dominant force. The techniques have a wide range of potential applications.

The new aspect of the work derives from the fact that the light beams are alternating rather than constant. Recent interest in optical trapping of free neutral atoms has produced an optical Earnshaw theorem² showing that steady radiation pressure traps cannot produce stable confinement, just as in the case of steady electrical fields and charged particles. However, just as ion traps can work very well with alternating fields, optical traps can work very well with alternating beams. Using an arrangement of mirrors and shutters to produce the appropriate beams, Ashkin and Dziedzic have confined and manipulated oil droplets of only 0.001 cm diameter for up to five hours. This was accomplished in the air, at atmospheric pressure, with no physical contact with the drop and no observable deterioration by heating.

Arthur Ashkin has been thinking about particle and atom trapping for a long time. In 1970, in his first paper on the subject³, he laid out the fundamental principles and suggested a number of applications. The attractive features of his technique for manipulating micron sized particles particles are the lack of physical contact, com-

plete control over motion and the lack of heating or disturbance by radiometric forces. The earliest experiments suggested the possibility of separation of particles by size or composition, as well as showing how to transport, levitate and handle them. Later in that same year, Ashkin described the possible application of these ideas to free neutral atoms⁴, a subject of much interest in recent years (reviewed in ref. 5).

Ashkin and colleagues have also succeeded in detaining free sodium atoms in a configuration of laser beams that produces viscous optical forces (S. Chu, personal communication). Our own group has recently accomplished the first real trapping of neutral atoms by confining laser-cooled sodium in a magnetic trap for more than 1 s (see accompanying article⁶).

Whereas it is clear that a new field of applied physics, involving the optical manipulation of free neutral atoms, micron size particles and other tiny objects, is being born, we cannot yet foresee the complete range of its applications. They will include materials processing, information storage, precision atomic spectroscopy, studies in classical mechanics and chaos, isotope separation, control of chemical reactions and manipulation of single cells or even macromolecules. Surely there will be many others.

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finely axially by a hyperbolic electrostatic potential distribution, and radially by the combined action of the electrostatic and magnetic fields. The ion motion comprised the cyclotron motion about the magnetic flux axis and the circular ($E \times B$) drift (or magnetron motion) each in the radial plane, together with the axial motion. Each may have a temperature associated with it. In the experiment described, the cyclotron and axial temperatures were less than 100 mK and the magnetron temperature less than 2 K (with the cooling laser on continuously). Some 2,000 ions were confined for many hours in the trap, with ion cloud diameters of 0.3 to 0.5 mm. The laser radiation was directed along the axis of the field and tuned to be slightly on the low frequency side of the absorption. The radiation not only cooled the axial motion but also coupled with the magnetron motion, and so could be used to compress (cool) the ion orbit.

Bollinger *et al.* report a measurement of the hyperfine ground-state transition frequency ν_1 , at its magnetic-field independent point (0.8149 T), of 303 MHz with an estimated standard deviation uncertainty of only 57 μHz (see Fig. 2). The experiment involved triple resonance, requiring first, 20 μW of 313 nm radiation to excite the $2S\text{-}2P$ optical transition; next 23.9 GHz microwaves to excite the indicated electron spin-flip transition; and finally, radio-frequency radiation to excite the transition ν_1 . The last was provided by a frequency synthesizer which was referenced to a hydrogen maser. The microwave radiation served to make the rate of excitation of the optical transition (and hence the observed fluorescence) sensitive to the upper level of the state pumped by the ν_1 line.

To excite and interrogate the ν_1 transition, a 0.5–2 s pulse of 303 MHz radiation was followed by a second 303 MHz pulse, 10 or 19 s later. This leads to a resonance some 25 mHz wide. It was only necessary to change the radiofrequency by millihertz steps over a 0.1 Hz range in order to scan the resonance. The optical and microwave signals were applied for 3 s before the first

pulse and after the second pulse, serving as state-preparation and state-sensing pulses, respectively.

Despite the cooling, the second-order Doppler-shift correction $-107 \mu\text{Hz}$ was still important. It arose from the heating of the ion cyclotron and axial motions to about 3 K during the 10 to 19 s that the laser was switched off and is probably due to small imperfections in the construction of the trap. It was also necessary to apply a correction of about $-6.1 \mu\text{Hz}$ to allow for the rotation of the earth (and ion trap) around the ion cloud.

The same team has gone on to use their apparatus to set 300-fold lower limits on the spatial anisotropy of inertia by performing a Hughes-Drever type of measurement (Prestage, J. D. *et al. Phys. Rev. Lett.* **54**, 2387; 1985). Their sensitivity was better than $50 \mu\text{Hz}$ to changes in the orientation of their $^9\text{Be}^+$ ions with respect to the Sun and Galaxy (see Gallop, J. C. & Petley, B. W. *Nature* **303**, 53; 1983). Various groups have explored certain clock transitions in $^{137}\text{Ba}^+$, $^{171}\text{Yb}^+$, $^{199}\text{Hg}^+$, $^{201}\text{Hg}^+$ and other ions, to the limits set by commercial caesium beam standards, by using different methods to cool the ions.

Other spectacular measurements with ion traps have been reported. Thus, the fluorescence signal from a single trapped ion has been seen (Neuhauser, R. *et al. Phys. Rev.* **22**, 1137; 1980) and, recently, Gabrielese, G. *et al. (Phys. Rev. Lett.* **54**, 537; 1985) reported an investigation of relativistic hysteresis effects in the cyclotron motion of a single electron that was trapped continuously over a period of ten months. In addition, the Dehmelt and Van Dyke group at Seattle, and others at Mainz and elsewhere, have made high precision measurements of the electron magnetic moment anomaly and the ratio of proton mass to electron mass.

There is no lack of narrow optical lines

that could be studied with trapped ions, but all have drawbacks and there are formidable experimental difficulties to be overcome. Thus, Q values in excess of 10^{14} are anticipated for the $^3\text{P}_0 - ^1\text{S}_0$ transition in Ti^+ , Al^+ or Ga^+ as well as in the $\text{D} \leftarrow \text{P} \leftarrow \text{S}$ Raman transition in Ba^+ , the $\text{S} \leftarrow \text{D}$ two-photon transition in Hg^+ or the $^3\text{P}_{3/2} \leftarrow ^3\text{P}_{1/2}$ transition in Pb^+ . Similarly, many high- Q transitions will be explored with trapped atoms — the $1\text{S} \rightarrow 2\text{S}$ transition in atomic hydrogen will be of particular interest. By contrast, the Q value of the current caesium frequency standards is in the region of 10^9 .

In the shorter term, the highest accuracies will be achieved on the measurements of fine and hyperfine structure transitions that lies at convenient radio and microwave frequencies. For the higher frequencies, the dye-laser technology must be improved to give wider frequency coverage and to use narrower line-widths. Also frequency multiplication into the visible region and beyond will have to be simplified and increased in accuracy.

As the techniques advance, we can expect to gain improved knowledge of atomic and ionic phenomena. One can even contemplate experiments involving new levels of sophistication and accuracy, such as parity conservation, gravitational interactions with atomic energy levels, Hughes-Drever type measurements, measurements of the properties of fundamental particles and, some day, the $1\text{S} \rightarrow 2\text{S}$ transition in anti-hydrogen. Moreover, we can expect greatly improved devices that will benefit technology as well as science — better frequency standards will almost certainly be one of the first gains. $\square$

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Archaeology

Man as part of the ecosystem

from Henry Cleere

SIR Mortimer Wheeler once described archaeology as a "humanistic science", and in that phrase he neatly encapsulated the confused, almost schizophrenic, view the discipline has of itself — a view, moreover, that is shared by those outside the field. Officially, the matter was resolved more than thirty years ago: government support to the Council for British Archaeology is paid through the British Academy rather than the Royal Society or one of the science research councils.

Whether or not archaeology can lay claim to be a science by virtue of its methodology remains a subject for debate, although it is undeniable that science now has an expanding role to play in archaeology. Of course, this is no recent phe-

nomenon: physical and biological sciences have contributed to the interpretation of archaeological data for many years. Accurate dating of archaeological material since 1945 has used techniques such as radiocarbon analysis, thermoluminescence and dendrochronology. The provenancing of stone, ceramic and metal artefacts has been improved immeasurably by advances such as neutron activation analysis, atomic absorption spectroscopy, electron-probe microanalysis and scanning electron microscopy, recently reviewed in ref. 1.

This, however, is science coming to the aid of archaeology in its more traditional guise — the classification and interpretation of human artefacts. Over the past

three decades there has been a significant change in archaeologists' perceptions of the range of materials available to them in their work. In modern excavations it is no longer enough to retrieve and examine artefacts and structures that are the result of direct human intervention: man is now seen as a component of an ecosystem, reacting with that ecosystem as it evolves over time and often profoundly and permanently modifying it to his own advantage. For the excavator it is essential to retrieve a wide spectrum of non-artefactual material from ancient contexts to recreate the environment of a site or of a region. This can help us to clarify the extent to which the environment has influenced the socio-economic development of a human community and how far man has wrought changes on that environment.

The new sub-discipline of 'environmental archaeology' is eclectic in the materials that form its basis. Plant remains may be macroscopic (from tree trunks to seeds) or microscopic (fossil pollen, phytoliths and diatoms). The presence or absence of pollens from the weeds of cultivation in the pollen diagram from a site can provide socio-economic information about a human community that artefactual assemblages may merely hint at or not represent at all. Palaeozoology embraces mammalian (and human) remains, fish and bird bones, insects of all kinds and molluscs, particularly land snails. Certain species of insects and land snails are highly stenotopic (adapted to a narrow range of habitats) and so their identification in an assemblage from an excavation can supply much specific information about a site and its environment. The correct identification of soils and sediments on and around early settlements provides important climatic and geomorphological date that can be crucial to the interpretation of a site and its community.

Several general studies on environmental archaeology have recently been published²⁻⁷. Some excavations have been carried out with the recovery of environmental data as a primary research objective: for example, half of the report⁸ on excavations at Farmoor, near Oxford, is concerned with the analysis and interpretation of environmental material. New techniques, such as froth flotation, have been developed to allow recovery of more complete and representative samples of biological material; the wealth of material has necessitated new analytical methods⁹.

The potential of environmental archaeology in enhancing the understanding of past societies is immense. However, that potential in Britain could not have been realized without the encouragement and financial assistance of the Ancient Monuments Laboratory of the Historic Buildings and Monuments Commission for England (formerly of the Department of the Environment), as part of its 'rescue' archaeology programme. An indication of the importance of that support is provided

by a recent government report¹⁰ which contains a "retrospective/prospective review" of the environmental archaeology of three regions (East Anglia, south-west England and northern England) and one historic town (York) that has been revealed by work sponsored by the laboratory over the past decade. These are the first regional overviews of past environments (as opposed to reports on the environments of specific sites) have been prepared. Although the reports stress problems resulting from lack of data, the information that has been assembled has produced several syntheses with profound implications for archaeology. For example, precise information on forest clearance and farming systems is emerging which, together with the results of aerial and ground surveys, radically changes the accepted view of the extent and nature of prehistoric and Roman settlements. Analysis of animal bone assemblages is throwing new light on dietary habits, animal husbandry and butchery practices. Analysis of large assemblages of human skeletal material, especially from urban cemeteries, is producing much invaluable — and often unexpected — palaeodemographic and palaeopathological data, which can be related directly to conventional archaeological data.

Whereas the enormous potential of environmental archaeology is clear, the

government report also emphasizes the inadequacy of funds to more than nibble at the edges of the subject, the material for which is becoming more voluminous with every excavation. Nevertheless, the report is a well-documented exposition of the value of treating human communities as components of a complex ecosystem rather than as isolated phenomena that do not interact with their environments. This is where the future lies for archaeology: let us hope that this will be realized by those who can make that future a reality. □

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Aggregation

Models of the sol – gel transition

from George Hentschel

ANYBODY who has seen a cold soup turn into jelly has witnessed a sol–gel transition. This is caused by the branching reactions that occur in the polymerization of polyfunctional monomers in a solvent. The gel point separates a sol phase, consisting of a distribution of growing but finite clusters in the liquid medium, from a gel phase where a macroscopically large branched network spans the system with fluid in its interstices. Yet, even today, it is not known whether monomer diffusion allows some reaction sites on the surface of a growing cluster, or whether one can treat all possible growth sites on an equal basis. A new kinetic-growth model, introduced by P. Meakin and Y. Termonia (*Phys. Rev. Lett.* **54**, 1083; 1985), now tackles possible diffusion-limited selection by combining aspects of both diffusion-limited aggregation (DLA) and the phenomenon of percolation. This is also critical because the dynamics of a fluid moving through a porous medium will cease to be determined by hydrodynamics when porosity is sufficiently ramified.

The model is a modification of the kinetic growth model of Z. Alexandrowicz for percolation clusters (*Phys. Lett.* **80A**, 284; 1980). Place a monomer at the centre S_0 of

a hypercubic lattice with coordination number f . Choose a probability P which represents the average fraction of new monomers joining the cluster per unit time. At time $t=1$, random variables z_i equally distributed in the interval $[0,1]$ are chosen for each nearest-neighbour site of S_0 . If $z_i < P$, a new monomer is placed at the site, whereas if $z_i > P$, the site is blocked for all times. This creates a set of new monomers S_1 . In the original model of Alexandrowicz, for all times $t \geq 2$ the algorithm described above is repeated for each new set of branch tips created at the previous step and not blocked. This identifies the sets $S_2, S_3, \dots, S_t$ of new monomers which join the growing cluster at each unit interval until for $P < P_c$ a point is reached where no new branch tips are created, as all possible growth sites are blocked. For $P > P_c$, the growth does not stop and a macroscopic cluster will span the lattice.

The novelty in the model of Meakin and Termonia is their contention that growth sites are not all created equal. As in DLA, the probability P_i for a branch tip on the surface to react with a monomer is larger than for one embedded in the cluster: monomers have to diffuse into the bulk to reach such a growth site and are likely to

have reacted before they can do so. To estimate the P_i for each new branch tip in this model, a large number of random walks from each vacant site is simulated and the fraction that escapes from the aggregate gives the relative probability of a monomer reaching that site. To find the absolute probabilities P_i , these relative probabilities have to be normalized so that an average fraction P is likely to be incorporated into the cluster at each step of the growth. Once the P_i have been estimated, each possible growth site is occupied or blocked by a new monomer according to whether a random number z_i chosen from the equal distribution $[0,1]$ is less than or greater than P_i . As in the case of percolation, a gel-like transition takes place at some critical rate of incorporation of monomers, P_c . The clusters are fractals with a fractal dimension which is that neither of DLA nor of percolation but, rather, more tenuous.

To see this model in the context of previous attempts to understand the gel point, note that diffusion has usually been neglected completely, and the rate-determining step assumed to be the reaction. All unreacted groups on a polyfunctional monomer were treated as equally likely to react, so there were no geometric constraints in the model. A Smoluchowski equation could be set up with reaction rates proportional to the product of unreacted species in each cluster, and solved for the cluster distribution. This model is soluble and exhibits a sol–gel transition. Though the model is satisfactory away from the gel point, the classical exponents do not agree with experimental results close to P_c .

One geometric constraint that can be introduced easily into the reaction process is to argue that some unreacted sites in the cluster may not be able to react because they are blocked, with some probability P by their neighbours. This would allow one to compare percolation and gelation to see whether they lie in the same universality class, as they both show transitions from finite to macroscopic spanning clusters. Unfortunately, percolation exponents are also in disagreement with experimental findings.

In the new model, the ease or difficulty of finding a partner in a unreacted group depends on the ability of a monomer to reach it, as well as blockage by its neighbours. The critical exponents of this model lie between those of classical theory and percolation. Meakin and Termonia compare their model with experimental tests and conclude that it agrees better than do either the percolation or the classical models, although discrepancies still exist. Clearly, monomer diffusion is important in clustering close to the sol–gel transition. □

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Extracted leaf protein in British agriculture

SIR — Extracted leaf protein (LP) is not made commercially in Britain and there is little research on it here. It is made commercially in Denmark, France, Italy, Japan, New Zealand, Poland, Spain, the United States and the Soviet Union. Because it is possible that it is we who are out of step, it seems timely to reconsider the arguments for LP production.

No one has questioned the argument, advanced more than 40 years ago and amply confirmed experimentally since, that the annual yield of protein in the form of LP can be greater than the yield of a protein concentrate by any other type of farming. Similarly, the demonstrations that LP can replace fishmeal or soy in pig and poultry diets, made 20 years ago in the National Institute for Research in Dairying and the Rowett Research Institute, have been confirmed in many other institutes. The residue remaining after protein extraction contains less water than any crop normally dried for conservation as winter feed for ruminants. Logically therefore, drying should be economical. There is ample evidence that the residue, because non-nitrogenous material is extracted as well as protein, still contains 1.5 to 2.0%N (in the dry matter) and that it makes good silage from which there is no wasteful and polluting effluent. This residue, whether fresh, dried, or ensiled is readily eaten by ruminants. Many experiments show that its feeding value is, weight for weight, as great as that of the original crop: this is presumably because more of the N in it is true protein and because comminution makes it more readily digestible.

One reason for the relative lack of interest in Britain in the use of LP as an animal feed, is the emphasis that has been put here on its value as a human food in countries with a humid climate and protein deficiency. As a result of successful human feeding trials, there are in these countries several production units managed by local enterprise rather than by sponsorship from outside. Its value and acceptability are now established. Even vegetarians in Britain usually eat diets containing an adequate amount of protein. There is therefore little need here for this application of LP. However, we feed pigs and poultry on imported fish meal, ground nut meal and soya: these could be largely replaced by home-produced LP. That substitution would not exacerbate the widely condemned overproduction of agricultural products. On the contrary, while economizing on imports, it would offer farmers an alternative to those crops which are now over-produced.

Another reason for lack of interest is that insufficient attention has been paid to the number of potential sources of LP. In early work at Rothamsted, a dozen suit-

able sources were described: the list could be extended, and it has been further extended in tropical countries. But in most countries where there is commercial production, attention is confined to grass mixtures and lucerne. The latter is agronomically attractive, but its juice froths inconveniently and, unlike some other species, it yields LP with an unappealing flavour. LP can also be extracted from by-products such as potato haulm, sugar beet tops and vegetable discards. If effort were put into collection, by-products could supply 100,000 tons of protein annually in Britain as well as a residue suitable for cattle feed.

Yet another reason for lack of interest is the use of unsuitable equipment and techniques in such large-scale trials as have been made. High-speed pulping equipment was used in early work at Rothamsted because we were sure it would work: it is still the most satisfactory equipment in agronomic studies. But pulping by slow-speed rubbing, or extrusion through a die, uses less power and is nearly as effective. If the pulp is less than 1 cm thick after pressing, and if pressure is maintained for a few seconds, juice expression is satisfactory with 1 to 2 kg force per cm² (100 to 200 kPa). The vast screw expellers, which have been used in some unsuccessful trials, are extravagant and perform poorly because tightly packed fibre impedes the outflow of protein-rich juice. There are simpler and cheaper methods for expressing juice: more should be devised, for this is the least satisfactory part of the process.

Lack of success in feeding trials with unfractionated juice was predictable. Its protein content is small and variable, its cation content is often excessive and, with some species of leaf, it contains unappealing or slightly toxic components. When a production routine has been established, there is no need to separate coagulated LP completely from the juice. If the coagulum is allowed to settle, the sediment has a relatively constant composition, and the supernatant carries away most of the deleterious material. Furthermore, if conservation is necessary because of an uneven supply of leaf, the compact sediment needs less of the preservative. Heating is preferable to any other method of coagulation. It is an illusion to regard it as expensive. Much of the necessary heat can, with a counter-current arrangement, be supplied by juice which has already been heated. If the pulper is driven by a diesel or steam engine on site, exhaust heat will be more than adequate for coagulation.

Without an estimate of costs, the place of LP in British agriculture cannot be assessed. Costs are obviously inflated when the equipment used is more elaborate and expensive than necessary, and when the value of the residual fibre as cattle feed, and the possible value of the

fluid which is separated from the coagulum, are not included in the calculation. So many factors tend to invalidate the gloomy conclusions drawn from trials which made inadequate use of existing knowledge and experience, that it was premature to decide that LP production would not be advantageous in Britain. No one has rebutted the basic propositions on which advocacy of LP production depends. The objective is sound, but execution has hitherto been faulty.

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Calmodulin and eukaryote evolution

SIR—Whilst reading the recent report by Sudhaker Babu *et al.*¹ on the three-dimensional structure of calmodulin, I was again impressed by this protein's ubiquitous role in eukaryotes. From cotton seed to rat testis it shows itself in a remarkably constant condition and appears to show that a similar ancestor must have been present at the dawn of eukaryote evolution.

Rightly or wrongly the next step is to review the prokaryotes for 'ancestral types' and *Escherichia coli*² has a contender in the form of the acyl/carrier protein which has a section resembling that of the E-F hand³ structure. Also, the antibiotic amphotomycin has been shown⁴ to have a calcium dependent action upon Gram-positive bacteria. Its structure⁵ shows it not to contain an E-F hand (it only contains 11 residues) but at least a second finger, that is, the loop of the helix-loop-helix described by Kretsinger and Barry³.

If the evolutionary link between *Streptomyces* antibiotic production and eukaryote calmodulin seems far-fetched, does this mean that the second finger of the E-F hand is the only decent way to employ amino acids in the capture of calcium ions?

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a rather technical character which are not provoked by articles or letters previously published (where Matters Arising remains appropriate).

A new approach to an absolute timescale from measurements of orbital cycles and sedimentary microrhythms

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When the sedimentary microrhythms of two groups of Jurassic zones forming part of the chronostratigraphical scale are analysed against recent radiometric age scales there is good agreement with present-day cycles resulting from the obliquity of the ecliptic. Sedimentary microrhythms resulting from orbital forcing can be used to establish either the relative or the true length of chronostratigraphical zones and thus provide a new approach towards an absolute timescale. Such a scale will be powerful in the analysis of other cycles and perturbations in the geological record.

THE inadequacies of the radiometric timescale for practical use in much of the Phanerozoic sedimentary record is well known. Reliance has always been placed on the relative timescale provided by evolutionary changes for local and international correlation and age determination. It is the biostratigraphical and chronostratigraphical scales¹ which form the standard for geological work on sedimentary rocks on land and in the oceans. But a numerical scale is obviously required for any useful analysis of sedimentary, tectonic and evolutionary processes. I show here how recognition of a causation for some sedimentary microrhythms in terms of the influence of orbital forcing cycles can provide a new approach to the establishment of an absolute timescale, using the evidence of the effect of such cycles in comparatively recent deep-sea cores²⁻⁴ and the recognition that sedimentary microrhythms common in the stratigraphical record^{5,6} may result from Milankovitch-type cycles⁷.

I will use two examples from the Jurassic to illustrate the technique. These examples enable integration with the chronostratigraphical scale. The Jurassic also demonstrates the essential problems with the current radiometric timescale. Table 1 lists several recent attempts to give the duration of the Jurassic and shows the uncertainty of each method. As to the finer divisions of the Jurassic used on radiometric scales, three approaches have been used recently. The first is to consider the 10 or 11 Jurassic chronostratigraphical stages to be of equal duration and to divide the number into the accepted duration for the system⁸. The second is to take the number of ammonite zones within a stage as the more reasonable estimate of its duration and to adjust stage length accordingly¹⁰. The third proposal^{11,12} is a weighted scheme using zonal and subzonal divisions. All three methods are based on the assumption that the evolutionary changes, which form the basis for stage, zonal and subzonal division, proceed at a uniform rate. Yet that is an hypothesis which a palaeontologist would wish to test, and the evidence presented here shows that is false.

Sedimentary microrhythms

There has been a wide recognition of rhythmic sedimentary events^{5,6,13} at a scale of ~0.2–3.0 m throughout the Phanerozoic and parts of the Precambrian. However, these rhythms are usually asymmetrical cycles of the ABCABC or ABCDABCD type, and there seemed little immediate relation to the regular symmetrical cycles (such as ABCDCBA) which one would expect from orbitally controlled patterns. Perhaps that is a reason why such interpretations have been regarded as speculations. Figure 1 illustrates how rhythmical or asymmetrical cycles may rise from symmetrical forcing oscillations when set against a regular trend in a changing parameter, for example, sea-level

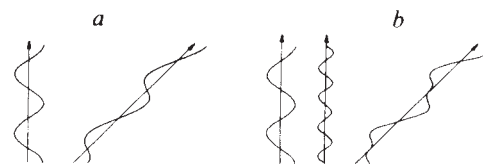


Fig. 1 Net effect of symmetrical oscillations and long-term trend giving a stepped effect of stasis and more sudden change. This model can be interpreted, for example, in temperature, sea-level, salinity, faunal abundance or climatic terms. *a*, Effect of one cycle; *b*, effect of two cycles, such as the tilt and precession cycles.

change against a subsiding sea floor, temperature oscillations against a progressive temperature change or climatic oscillations against a climatic trend. The simple patterns illustrated in Fig. 1 show a period of rapid change followed by relative stasis of the type needed to explain many microrhythms. Such a situation may be provided by a combination of the Milankovitch-type orbital oscillations⁷. More complex interpenetration of several cycles and combinations of cycles with different control could give very elaborate effects. In such cases, different amplitudes and wavelengths can give harmonics and varieties of pattern which might, at first glance, seem bewildering, but which would have simple primary cause⁹. This is illustrated in Figs 1 and 5 which show how the effects of simple cycles which can be resolved using Fourier transforms.

Many microrhythms in the geological record result from quite different causes, for example, turbidite flows resulting from continental shelf and slope instability, nearshore storm events, periodic fault movements or spasmodic volcanicity. Orbitally related phenomena may be best studied in deeper sedimentary basins away from such phenomena. To proceed with the construction of the new timescale only a few sequences well-related to the chronostratigraphical scale are needed, then normal biostratigraphical techniques can be used.

Table 1 Estimates of the duration of the Jurassic period based on recent radiometric timescales

Ref.	Date	Duration of Jurassic (Myr)
Van Hinte ³⁵	1976	55–60
Harland <i>et al.</i> ³⁶	1982	69
Odin ³⁷	1982	74
Kent & Gradstein (in Palmer ³⁴)	1982	64
Snelling ¹¹	1985	70

Jurassic examples

To demonstrate a possible relation between sedimentary micro-rhythms and orbital patterns two cases will be considered from English Jurassic sequences which form part of the chronostratigraphical scale, one from the Lower and one from the Upper Jurassic. Figure 2 illustrates the typical sequence for the south of England along the Dorset coast. The large-scale rhythmicity shown is not of concern here but was appreciated as early as 1822 (ref. 14) and has been described in detail elsewhere¹⁵⁻¹⁷. Each of these major rhythms usually show finer-scale micro-rhythms, the immediate cause of which have been investigated extensively in terms of small sea-level changes and anoxic events¹⁶. Two examples will be treated here, part of the Lower Lias and part of the Kimmeridge Clay, chosen because their unambiguous relation to the chronostratigraphical scale. The initial postulate for this study is that the observed regular small-scale rhythmicity indicates the overprinting of a periodic environmental change.

The careful measurements of the Lower Lias microrhythms of Lyme Regis, Dorset by Lang form the basis for the calculations given here^{18,19}. The microrhythms typically comprise a thin laminated, dark, organic-rich shale followed by lighter coloured calcareous shale with bioturbated limestone beds at the top²⁰⁻²². Not all units are developed and higher in the succession the limestone units are often lost. The succession of these microrhythms and their correlation with the standard chronostratigraphical scale²³ is illustrated on Fig. 3a using Lang's data and terminology for bed numbers. The Lower Lias sequence is nearshore and could be expected to be incomplete²³; it represents a deepening sequence from possible supratidal stromatolitic Upper Triassic beds below. Some data from Somerset²⁴ are also considered.

The broad features of the Upper Jurassic Kimmeridge Clay have been described²⁵⁻²⁷. The typical microrhythms comprise alternations of well-laminated oil shale and/or laminated organic-rich shale with more calcareous, lighter coloured shale or mudrock. This sequence is interrupted by dolomitic or coccolith limestone bands, termed stone bands, which form convenient markers. Immediately above these markers microrhythms are often not clearly seen. The Blackstone, or Kimmeridge Oil Shale, is the best developed and thickest oil shale. This and associated oil shales are thought to be the main source of oil in the North Sea fields and for the nearby Wytch Farm field (Fig. 3b)²⁶. An illustration of a cliff section showing some of these microrhythms is given on Fig. 4b.

Analysis of microrhythm data

Only Arkell²⁸ in this century has had sufficient detailed biostratigraphical command of the whole Jurassic to give judgement on zones which might have reasonable balance throughout the system. He used 58 zones, excluding the non-marine Lulworth Beds to which we may assign two zones for calculation, giving 60 in all. Arkell's subdivision of the Kimmeridge Clay was

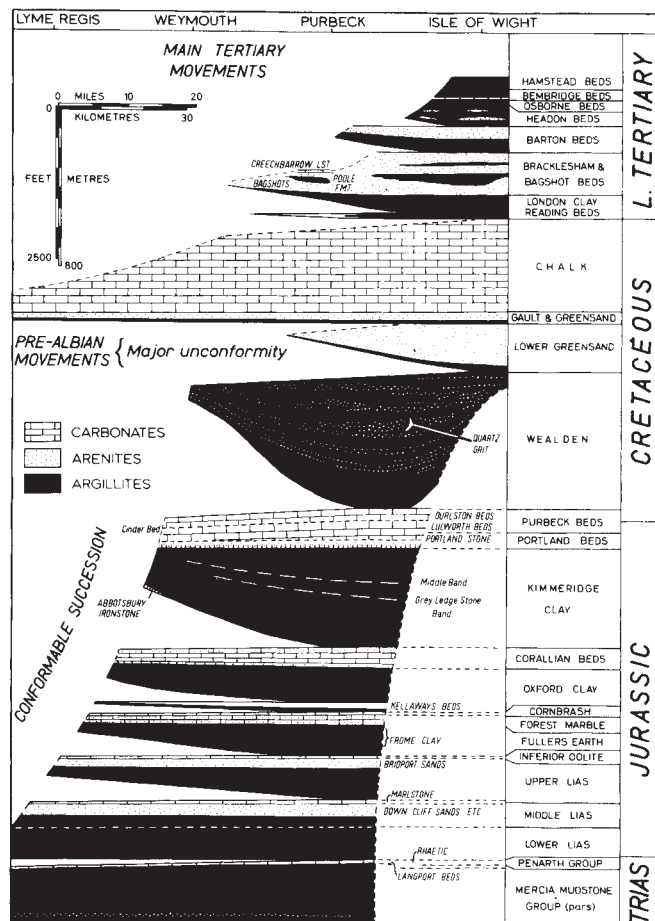


Fig. 2 Mesozoic and Tertiary sequence of the Dorset coast drawn to emphasize the large-scale rhythmicity of the succession and to indicate the levels of the sequences discussed in greater detail (after ref. 14).

provisional and if revisions of the sequence between the Mutabilis and Pectinatis Zones^{25,26} are accepted, the total of zones is not altered but all the Kimmeridge Clay data on Fig. 3b can be used. More recent lists give up to 74 zones²³; if this value is preferred other calculations may be done, but I feel that this introduces too much relative subdivision at certain levels.

The four earliest Jurassic zones used here²³ compare with the first three of Arkell's scheme²⁸ to estimate zone duration. Using Lang's limestone units for the first approximation (Fig. 3a) these give the following crude numbers of microrhythms for each zone. (Shown in parentheses are interpolated rhythms which have been added for use in the calculations.) Planorbis Zone (beds H25-H56), 16(6); Liasicus Zone (beds H57-H83), 13(6, assuming six rhythms when complete between each of refs 2-4); Angulata Zone (beds H84-20), 19(5); Bucklandi Zone (beds 21-46), 13 (14). This gives a total of 91 for the four zones (three in Arkell's scheme²⁸). No estimate is made for the Semicostatum Zone, but rhythmicity clearly continues (Fig. 3a).

Data from Somerset²⁴ suggest that at Doniford Bay the following values hold: Planorbis Zone (beds 14-42), 10; Liasicus Zone (beds 43-69), at least 18; Angulata Zone (beds 80-145), 39 (or 40 at Blue Ben); Bucklandi Zone (beds 146-203), 27. This gives a total of 94 microrhythms. But there is considerable subjectivity in the estimation of these numbers and some discrepancy between them and those of Dorset. The Lias evidence is used in the absence of better data, but a more thorough regional and international study should provide more reliable values.

The Kimmeridge Clay microrhythms used for the calculations are shown in Fig. 3b where interpolated levels are the numbers

Table 2 Estimates of the time duration of microrhythms of the Lower Lias (Planorbis to Bucklandi Zones) and Kimmeridge Clay (Autissioderensis to Wheatleyensis Zones)

Duration of Jurassic (Myr)	Duration of each zone (Myr)	Duration of Lias rhythms (kyr)	Duration of Kimmeridge rhythms (kyr)	Average (kyr)
60	1.00	33.0	40.8	36.9
65	1.08	35.6	44.1	39.8
70	1.17	38.6	47.8	43.2
75	1.25	41.2	54.9	48.1

Several possible duration times are used for the Jurassic, and it is assumed that the zones are typical in length for the Jurassic using a slight modification of Arkell's zonal scheme²⁸ (see text for details and the effect of other assumptions).

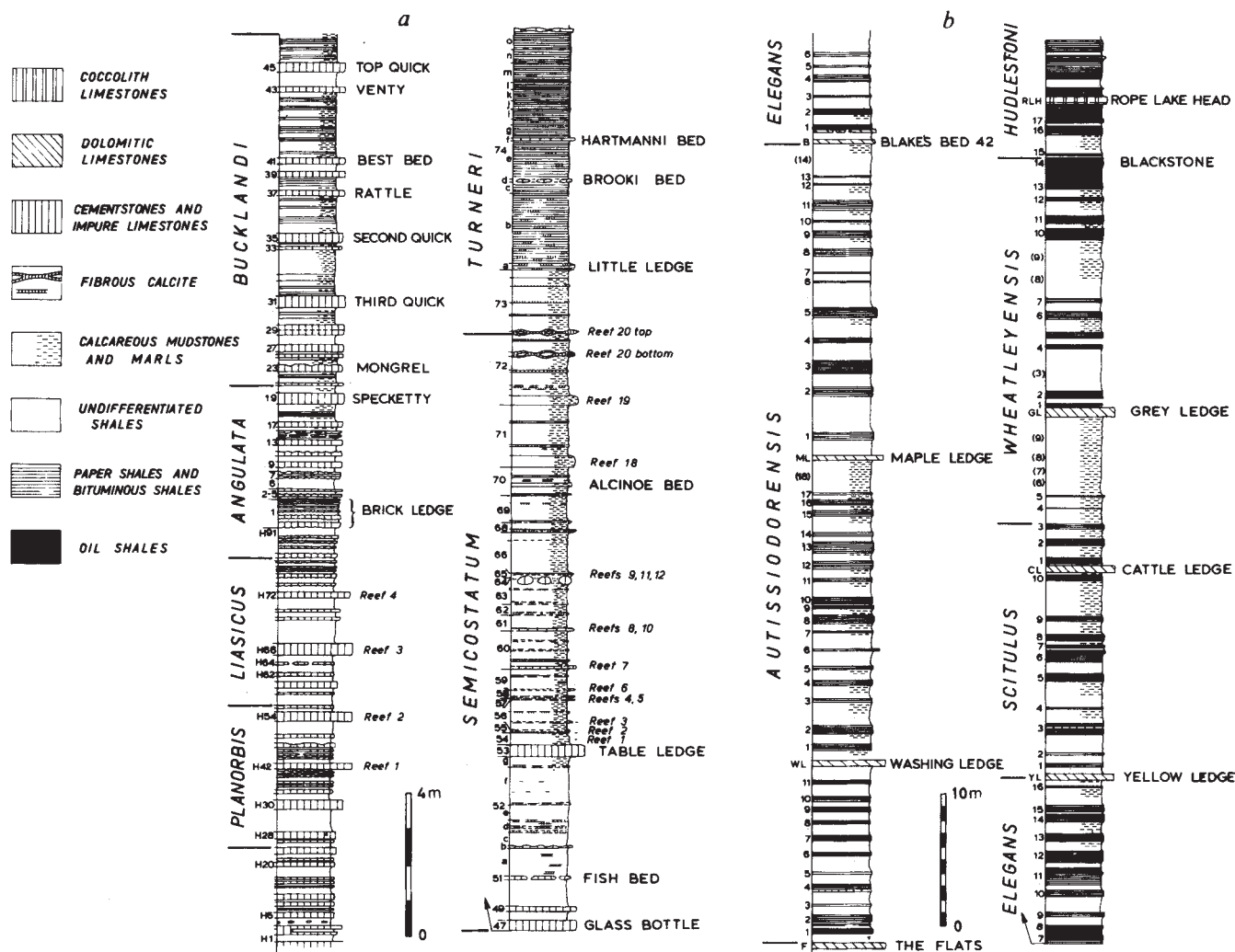


Fig. 3 Detailed sequences from the Jurassic of the Dorset coast illustrating sedimentary microrhythms. *a*, Part of the Lower Lias of the coast near Lyme Regis based on detailed descriptions and bed numbers of Lang^{18,19}. The base of the Jurassic and Hettangian Stage is taken where *Psiloceras* enters in bed H25 and the base of the Sinemurian is taken at Bed 21. Numbers with prefix H refer to the section west of Seveh Rock Point, those without prefix extend from the Point eastward to the foreshore below Black Ven. *b*, The section near Kimmeridge between Kimmeridge Bay and Clavell's Hard based on published data of Cox and Gallois²⁵. The bed numbering is new and the marker beds are abbreviated so that they form a prefix for the bed numbers given above them up to the next marker bed. A numbering system is given for reference which refers to units above particular marker beds. Thus, ML5 refers to fifth organic-rich shale above the Maple Ledge Stone Band. The boundary between the Lower and Upper Kimmeridgian (*sensu anglico*), and between the Kimmeridgian (*sensu gallico*) and Tithonian stages is usually drawn at Blake's Bed 42 (B).

in parentheses. Zonal assignments are those of a recent revision²⁶. The numbers used are as follows: Autissiodorensis Zone (F1 to MP14), 45; Elegans Zone (B to B16), 17; Scitulus Zone (YL to CL3), 15; Wheatleyensis Zone (CL4 to GL14), 21. This gives a total of 98 microrhythms. Table 2 gives calculations of the time for each microrhythm using only the Dorset data.

Interpretation

There are several uncertainties in the values used, of the period taken for the length of the Jurassic, of the number of Jurassic zones and of the assumption that the eight zones used are a fair sample for the Jurassic. Nevertheless, the agreement of an average of the microrhythm period and an average of current estimates of the duration of the Jurassic with the value of 41 kyr (ref. 4) which is the oscillation resulting from the obliquity of the ecliptic at present is very close (Table 2), perhaps fortuitously so. The differences between the Lias and Kimmeridge results could be due to different weighting in the true zonal periods involved. I suggest that the obliquity cycle may be the primary cause of the dominant rhythmicity. If the obliquity periodicity has changed little through time, then estimates of the duration

of the zones, using the 41-kyr value give the following results (in Myr): Planorbis Zone, 0.86; Liasicus Zone, 0.78; Angulata Zone, 0.94; Bucklandi Zone, 1.11; Autissiodorensis Zone, 1.84; Elegans Zone, 0.7; Scitulus Zone, 0.62; Wheatleyensis Zone, 0.86. For the present, these values should only be taken, in their blocks of four, as relative times which may be assigned to the zones. Note that if the 41-kyr cycle did hold in the Jurassic these values indicate (within the limit of sampling) which duration is the more probable for the Jurassic. The present evidence would favour the Cambridge scale³⁶, but other time and zonal scales give different results because of unbalanced zonal weighting. Thus, Hallam²⁹ gives the Kimmeridgian (*sensu gallico*) and Tithonian 22 zones and a span of 11 Myr, which would give a period of 20.4 kyr for the Kimmeridge microrhythms. Data from the same review would give an average of 62.5 kyr for the Hettangian microrhythms (giving, as expected, an average of ~41 kyr). The true value will only be approached when relative lengths for zones, using the technique proposed here, are assembled throughout the Jurassic. But do such errors explain the diversity of estimates of microrhythm duration in other parts of the stratigraphical column?

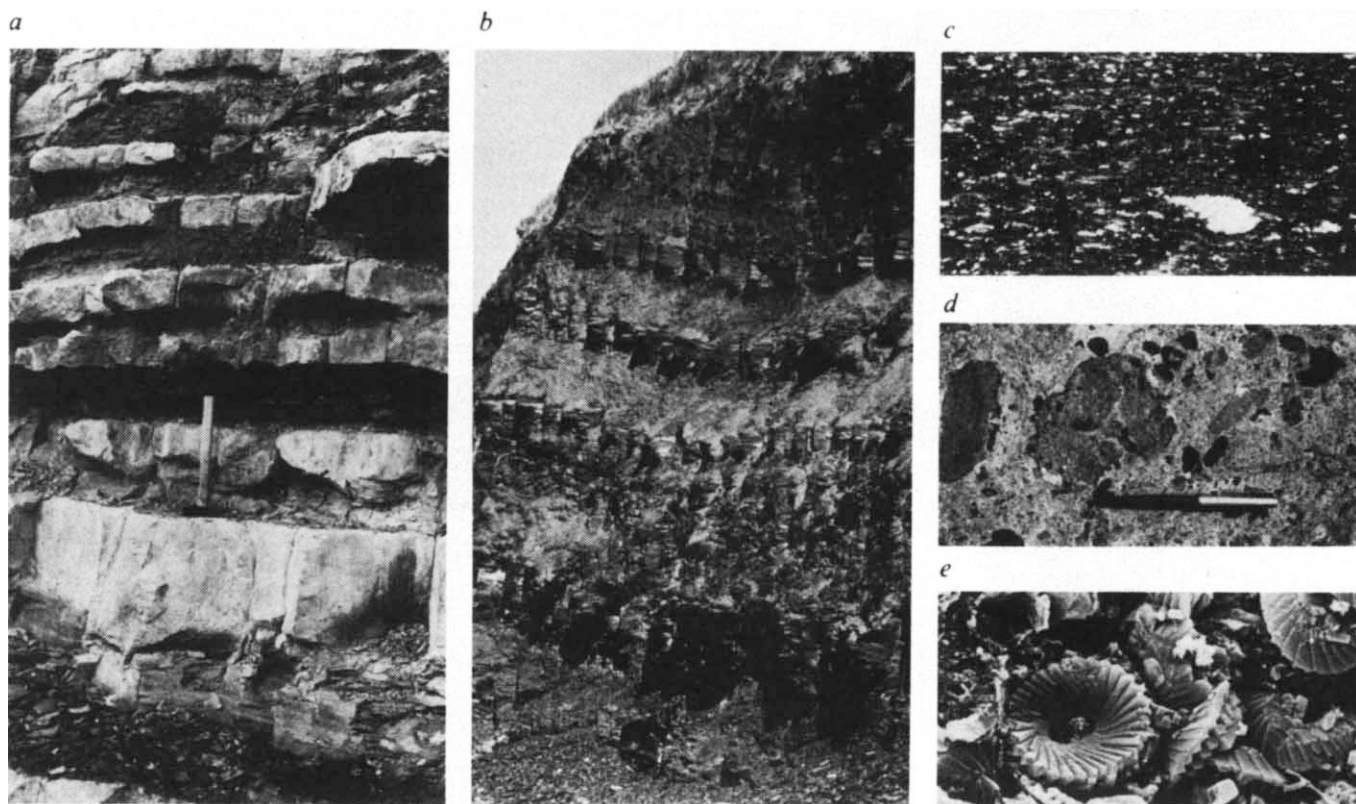


Fig. 4 Features of Dorset Jurassic sedimentary microrhythms. *a*, Part of the Lower Lias succession in Chippel Bay, west of Lyme Regis. The hammer is on the unit called Specketty (Bed 19). *b*, A cliff profile in Kimmeridge Bay, just west of Gaultier Gap, showing the weathering profile of microrhythms above The Washing Ledge Stone Band (Bed WL2 is at the base of the cliff). Four zones are represented (Autissiodorensis to Wheatleyensis). *c*, Section of The Blackstone (Bed GL14) illustrating clasts within the spore-rich oil shale. *d*, Debris deposits in the White Stone Band west of Freshwater Steps, Kimmeridge. Pen, 13 cm long. *e*, Stereoscan photograph of a specimen from the Rope Lake Head Stone Band showing well-preserved coccoliths without a trace of biodegradation. Width of photograph, 15 μm .

The obliquity cycle operates by the shifting of latitude and the microrhythms may be explained, for example, by small climatic shift^{29,30}. Thus, oscillations in and out of the tropical forest belt might explain the organic-rich, anoxic phase and the hitherto anomalous evidence for turbid flow and coarse debris within parts of oil shales and organic-rich shales in the Dorset Jurassic (Fig. 4c, d). This provides a better model than reliance on sea-level changes, although these may be involved in some cases. Perhaps the most promising model is that invoking cold-water benthic versus warm-water planktonic regimes³¹. Although the amount of variation in tilt is likely to be less in the past because of the greater number of days in the year, it seems reasonable to suppose, as a working hypothesis, that the period of the obliquity cycle is probably constant.

Clearly, there are other oscillations that may affect the system, both at greater and smaller frequencies. Thus, the Lias Beds H30 to H66 show three major units forming the reefs, each separated by five minor limestones, which may indicate six oscillations between the maxima of a larger cycle. The Lias sequence discussed here has been the subject of Walsh power-spectra analysis, suggesting orbital-precession (19 and 23 kyr) and a 31-kyr obliquity cycle in addition to those mentioned³⁰. These values depend on the assumption of 1-Myr zones and of sedimentation rates. The effect of such cycles seems limited to the introduction of irregularities in the rhythmic pattern. In the group of zones considered here, limestones comprise ~25% and argillites the remainder. A single, probably varved, limestone from above the Brick Ledge gives a 'varve' average of 850 μm . Estimates for varves in organic-rich levels have been given²⁰ as 9–17 μm and the average, 14 μm , may be used for calculation. Given a total thickness of 25.6 m, these values suggest a depositional time of 1.5 Myr when the expected duration for average zones would be ~4 Myr giving a total of time hiatus of 62.5%

comparable with another recent estimate³⁰ of 70%. But I suggest here that the major rhythmicity signature, estimated as 41 kyr, is probably nearly complete and the loss occurs in individual rhythms.

The Kimmeridge data suggest a repetition of coccolith limestones or dolomitic limestones with a cycle, perhaps a harmonic of about twice or three times that shown in Lias Beds H30 to H66 referred to above. Such signatures of oscillation interaction may prove to be time definitive. A computer model, from a program by R. Middleton, is shown in Fig. 5, and will be used to illustrate an interpretation of the Kimmeridge Clay data. The dominant periodicity (41 kyr) is that obtained by present calculations. The expected 100-kyr cycle is added weakly as it shows no very obvious visual effect. The largest period (taken at 451 kyr) is suggested by the stone band position (F to F11, YL to YL10, CL to CL(9) taken as about 11 times the supposed obliquity cycle) and is used to illustrate the fact that the different controlling oscillations may operate differently. Thus, the points marked *a* on Fig. 5 show times when climatic shift crosses the environmental threshold, enabling the production of organic-rich or oil shales, the threshold being indicated by the straight line given an arbitrary trend. This threshold will be expected to vary from place to place and, in particular, anoxia will reduce in shallower and more agitated waters. At the points marked *b* in Fig. 5, however, the threshold is not crossed and the rhythms may not be obvious in the sediments and they will not show as organic-rich shales. This is the justification for the interpolations used in the calculations (Fig. 3a, b). A cycle of larger wavelength affecting the shape of the orbit, similar to the present-day 100-kyr cycle (but given on the model a 451-kyr periodicity), could result in periods of much higher global insolation and hence, for example, in more extreme temperatures. This might explain periods of coccolith blooms (Fig. 4e) and the periodic formation

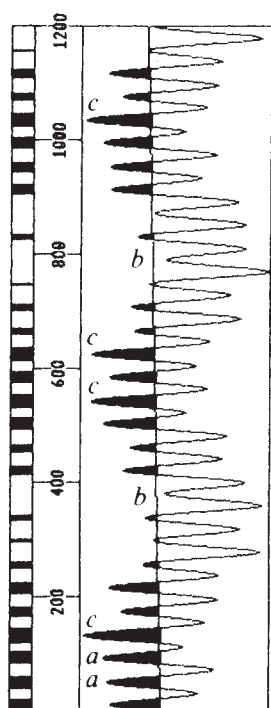


Fig. 5 Computer simulation of microrhythm patterns using 41 kyr as the main obliquity periodicity with amplitude taken as unity. Additional oscillations arbitrarily introduced at wavelengths of 451 and 100 kyr with amplitudes of 0.6 and 0.3, respectively. The vertical column indicates conditions when organic-rich shales may be produced if the trend line represents an environmental threshold for their formation. Scale in kyr.

of the marker stone bands, which would be under quite a different control from the obliquity latitudinal shift. Two such cycles could interpenetrate in their magnitude and in their effect. In this interpretation, it is assumed that the carbonate marls necessary for the production of the diagenetic dolomitic stone bands³² arise from pelagic carbonates formed on occasions similar to those producing coccolith blooms. A small portion of this sequence, including the Blackstone, has been the basis of Fourier analysis using detailed chemical data³³ and assumptions of sedimentation rate has given evidence of smaller-scale cycles. When the chronostratigraphical scale for the Jurassic is calibrated numerically by the summation of relative duration data, Walsh power spectral analysis and Fourier analysis should enable the provision of very precise astronomical data on planetary perturbations.

At a larger scale are the rhythms producing widespread anoxia in the Jurassic in the Toarcian, Callovian and mid-

Kimmeridgian (*sensu anglico*) but whether they have a regular period has still to be demonstrated.

The many examples of rhythmicity described in other parts of the Jurassic and in other stratigraphical systems need to be studied to contribute to the calibration of the timescale and the establishment of a numerical scale linked to the chronostratigraphical scale along the lines suggested here for the Jurassic. It should be emphasized, as is illustrated by Table 2, that even in the relatively well-known Jurassic, bizarre results may follow the choice of an inaccurate radiometric time scale. Subject to the monitoring suggested here, orbital forcing patterns may become the more reliable tool.

Conclusions

If the microrhythms discussed are periodic then, whatever their cause, they provide a better means of assessing the relative lengths of zones for purposes of subdividing the radiometric timescale than the present assumption that stages, zones or subzones are of equal duration.

Assuming values for the average duration of blocks of Jurassic zones based on current radiometric scales, the period for the microrhythms discussed accords well with the present value (41 kyr) for the period resulting from the obliquity of the ecliptic. If this is the primary cause of the sedimentary rhythmicity then it can be used to estimate the absolute length of zones. But other values result from different premises.

It is argued that either relative methods or, eventually, orbital cycle methods can be used to build up a precise date in years for zonal sequences so that the chronostratigraphical scale can become a numerical means of estimating geological processes quantitatively.

I suggest that such a quantitative scale is the only convincing way in which larger-scale stratigraphical and palaeontological perturbations can be dated and interpreted.

I conclude that the assumption made in subdividing the existing radiometric timescale that zones or subzones are of equal duration is false.

If the primary cause of the main rhythms examined is periodicity in the angle of the Earth's tilt then the interpretation of them in terms of slight shifts of climatic belts becomes reasonable. Other rhythms may have quite different orbital forcing controls, such as global insolation changes. The examples given are intended to illustrate a technique rather than to provide final values or interpretations for the phenomena discussed.

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Amino-acid substitutions at codon 13 of the *N-ras* oncogene in human acute myeloid leukaemia

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DNA from four out of five patients with acute myeloid leukaemia (AML) tested by an *in vivo* selection assay in nude mice using transfected mouse NIH 3T3 cells were found to contain an activated *N-ras* oncogene. Using a set of synthetic oligonucleotide probes, we have detected a mutation at codon 13 in all four genes. The same codon is mutated in an additional AML DNA that is positive in the focus-formation assay on 3T3 cells. DNA from the peripheral blood of one patient in remission does not contain a codon 13 mutation.

THE use of focus assays to detect morphological transformation of NIH 3T3 cells in DNA transfection experiments has shown that between 10 and 30% of human tumours contain altered forms of either the c-Ha-ras-1 gene, the c-Ki-ras-2 gene or the *N-ras* gene. These genes encode GTP-binding proteins of 188 and 189 amino acids that are located at the inner surface of the cell membrane and which are evolutionarily highly conserved^{1,2}. In the 15 or so cases of altered *ras* genes in human tumours that have been analysed so far, activation has been shown to result in a single base pair mutation which leads to the substitution of either a glycine at amino acid 12 or glutamine at amino acid 61 (refs 3–17). However, *in vitro* mutagenesis experiments have shown that mutations at amino acids 13, 59 and 63 can also lead to transforming activity¹⁸.

We have studied the presence of activated *ras* genes in acute myeloid leukaemia (AML), a disease characterized by abnormal proliferation and differentiation of cells of the myeloid, monocytic and erythroid lineage. This disease is subdivided into six groups (FAB M1–M6; ref. 19) according to which immature cell is dominant in the population. Using a direct *in vivo* selection assay in nude mice of transfected 3T3 cells²⁰, we find that four out of five AML DNAs tested contain an activated *N-ras* gene. An altered *N-ras* oncogene was also detected in another AML sample using the focus-formation assay. We analysed these activated *N-ras* genes for the presence of mutations using a set of synthetic oligonucleotide probes and report here that all five activated *N-ras* genes are found to contain a mutation at codon 13.

Transfection of NIH 3T3 cells

In initial experiments using focus formation on NIH 3T3 cells as an assay for the transfer of transforming genes, DNAs prepared from five samples of AML cells were found to have low, if any, transforming activities (D.T. and C.J.M., unpublished results). However, in a different laboratory one out of three AML DNA samples was found to have transforming activity³⁸. Because focus formation may not detect all transforming genes (for example, some alterations to *ras* genes lead to only a minimal change in morphology of the transformed cells²¹), we sought to increase the sensitivity of the transformation assay. Previously, Blair *et al.*²⁰ have shown that direct *in vivo* selection of transformed cells can be used as an assay for the transforming genes in transfected cells. As an additional step to ensure that all the injected cells had been transfected with donor DNA, DNA samples were co-transfected with the plasmid pSV2Neo (ref. 22) and the transfected cells selected in antibiotic G418. A similar approach has been described by Fasano *et al.*²³, but we injected a smaller number of cells than these authors. We found that tumours arose 30–55 days after inoculations of NIH 3T3 cells transfected with four out of five preparations of DNA from AML samples (AML 33, 49, 73, 77). Of 15 other human tumour DNA samples tested in the same way, 10 failed to give any tumours, even within 90 days, whereas 5 other samples gave tumours 32–80 days after inoculation of transfected cells (Table 1). Normal DNA prepared from either mouse livers or human lymphocytes gave a very low incidence of tumours—0/3

Table 1 Co-transfection and nude mouse tumorigenicity of AML DNAs

DNA + pSV2Neo	FAB classification of AML	Primary tumour incidence per co-transfection of 20 µg DNA	Latent period (days)	Secondary tumour incidence per co-transfection	Latent period (days)
Normal mouse DNA		3/28	94	NT	
Normal human		0/3			
HT1080		3/3, 1/3	30–70	NT	
AML 33	M1	1/3	50	3/4	39
AML 49	M5	1/3, 2/3, 1/3	37	3/3	27
AML 73	M2	1/3	30	1/4	34
AML 77	M4	1/3	55	NT	
AML 50	M5	0/3	90	NT	
15 Human tumours (non-AML)		10 × 0/3, 4 × 1/3, 1 × 2/3	32–80	NT	

20 µg Cellular DNA and 300 ng pSV2Neo were precipitated with calcium phosphate into each of three 60-mm plates seeded 1 day previously with 2×10^5 NIH 3T3 cells; 22–24 h later, the co-precipitate was washed off and the cells incubated in normal growth medium (Dulbecco's modified Eagle's medium + 10% calf serum) for 20 h. Each dish was then trypsinized and seeded to a 150-mm dish containing selective medium with 1 mg ml⁻¹ G418. After 7–10 days of growth in selective medium, each dish contained $1\text{--}1.5 \times 10^3$ G418^R colonies and a total of $3\text{--}4 \times 10^6$ cells. 1.5×10^6 cells were then injected into each of two inguinal subcutaneous sites of *nu/nu* mice. NT, not tested.

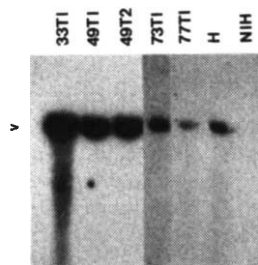


Fig. 1 Southern blot analysis to show the presence of human *N-ras* in NIH 3T3 transfectants from AML DNAs. 33T1, tumour in nude mice derived from co-transfection of NIH 3T3 cells with pSV2Neo and AML 33 DNA; 49T1 and 49T2, two tumours that arose from separate experiments with AML 49 DNA; 73T1 and 77T1, tumours that arose from experiments with AML 73 and 77 DNA, respectively. Control samples are DNAs from normal human fetal lung and NIH 3T3 cells. In each lane 5 μ g *Eco*RI-digested DNA was separated. Arrow, 8.8 kb.

Methods. DNAs were digested with *Eco*RI, electrophoresed in a horizontal (0.7%) agarose gel and blotted to nitrocellulose. The filter was hybridized (5 $\times$ SSC, 20 mM sodium phosphate, 5 $\times$ Denhardt's solution, 10% dextran sulphate, 50 μ g ml⁻¹ denatured salmon sperm DNA, 50% formamide) at 42°C for 16 h with 10⁸ c.p.m. ml⁻¹ of a ³²P-labelled probe obtained by nick-translation of the 800-bp *Eco*RI/*Sst*I fragment of *N-ras* contained in pAT8.8 (ref. 24). This fragment does not hybridize to mouse DNA and detects a single 8.8-kilobase (kb) fragment in *Eco*RI digests of human DNA. After hybridization the filters were washed twice in 0.1 $\times$ SSC, 0.1% SDS at 60°C and then exposed to Kodak XAR-5 film at -70°C with intensifying screens.

(human) and 3/28 (mouse)—and any tumours which did appear only arose 90 days after inoculation. DNA prepared from HT1080 cells, known to contain an activated *N-ras* oncogene²⁴, produced tumours 30–70 days after inoculation in 4/6 co-transfections. The transforming activity detected in the first transfection with AML DNAs could be passaged serially with similar or slightly greater efficiencies (Table 1).

To examine whether any of the tumours that arose in nude mice resulted from the acquisition of a human *ras* oncogene, DNA prepared from the tumours was analysed by Southern blotting with probes for *N-ras*, *Ha-ras* and *Ki-ras*. It was found that DNA prepared from each of the primary transfectants from the four AML DNAs detected by *in vivo* selection (Fig. 1) and the one detected by the focus assay contained the human *N-ras* gene. In some, but not all, transfectants the human *N-ras* gene was amplified 20–30-fold. There was no evidence for transfer of any of the other *ras* oncogenes, and tumours that arose after co-transfection of non-AML DNA did not contain human *ras* genes (data not shown).

Mutations at codon 13

To analyse the transfected *N-ras* genes in the NIH 3T3 transfectants derived from AML DNAs, we used synthetic 20-mer oligonucleotide probes. Using these probes we are able to detect single base pair (bp) substitutions in the *N-ras* gene based on the fact that a fully matched hybrid between the oligomers and genomic DNA is more stable than a 1-bp mismatched hybrid²⁵. For the positive detection of mutations, we have synthesized groups of oligomers varying at one position in a codon (see Fig. 2). One oligomer of each group will then hybridize to form a fully matched hybrid with each of the possible mutations in a base pair. For example, the oligomer group N12-p2 consists of three oligomers, one of which will form a fully matched hybrid with an *N-ras* gene mutated at the second base of codon 12.

When *Pst*I-digested DNA from four AML transfectants (33T, 49T, 77T and 1T) was hybridized to the probe corresponding to the sequence of the normal *N-ras* gene around codon 61

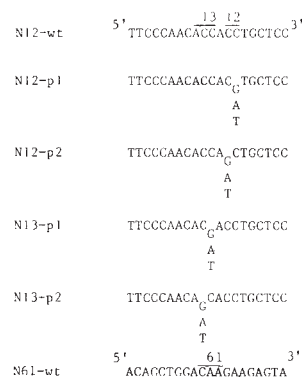


Fig. 2 Sequence of the 20-mer probes used to detect mutations in codon 12, codon 13 and codon 61 of the *N-ras* gene. Sequences are derived from Taparowski *et al.*¹².

Methods. The N12 and N13 probes were synthesized using as template chemically synthesized 20-mer and an 8-mer primer complementary to the 3' end of the 20-mer. This primer-template complex was incubated with [α -³²P]dCTP (3,000 Ci mmol⁻¹; Amersham), cold dGTP, dATP and TTP, and DNA polymerase I as described by Bos *et al.*¹⁷. The 8-mer primer carried a 5'-terminal phosphate group which enabled us to separate the labelled oligomer from the unphosphorylated template on a polyacrylamide urea gel. The N61 probe was synthesized similarly but now using [α -³²P]dATP (see ref. 17). The oligonucleotides were prepared by a phosphotriester approach³⁷ using a fully automatic synthesizer (Biosearch SAM-I).

(N61-wt) and washed under conditions in which only a fully matched hybrid is stable, a strongly hybridizing signal was seen (Fig. 3A). In two transfectants (49T and 33T) the signal was very intense, showing that the *N-ras* genes were highly amplified (see also Fig. 1). No hybridization was seen with the oligomer groups corresponding to mutations at codon 61, indicating that none of the *N-ras* genes in the transfectants contained mutations at codon 61 (data not shown). In contrast, the oligomer corresponding to the normal sequence of the *N-ras* gene at codon 12 (N12-wt) failed to form a fully matched hybrid with any of the AML transfectant DNAs (Fig. 3B), although it did hybridize to human cellular DNAs containing single-copy representations of the wild-type sequences at position 12 (lanes a, b). However, when the transfectant DNAs were hybridized with the groups of probes corresponding to mutations at each of the base pairs of codon 12 (N12-p1, N12-p2), fully matched hybrids were not detected (Fig. 3C, D).

The absence of fully matched hybrids with either normal or mutant probes led us to investigate whether any mutations occurred in the sequence surrounding codon 12. Recently, Fasano *et al.*¹⁸ have shown by *in vitro* mutagenesis that mutations in codon 13 (Gly) can lead to transforming activity of the c-Ha-*ras*-1 gene. Therefore, we analysed the transfectant DNAs by hybridization with probes (N13-p1 and N13-p2) that could recognize mutations at the first or second position of codon 13. The oligomer group N13-p2 hybridizes with each of the AML transfectant DNAs (Fig. 3F). Those transfectants with the highest copy number of the *N-ras* gene showed the greatest signal with the N13-p2 probe, but hybridization was also seen in a transfectant (77T1) that only contains one or a few copies of *N-ras* (compare Figs 1 and 3F), whereas no hybridization was detected with control DNAs having the wild-type sequence (Fig. 3, lanes a, b). Thus, we conclude that the N13-p2 probe forms a fully matched hybrid with the AML transfectant DNAs. No hybridization was detected with the N13-p1 probe to the transfectant or control DNAs (Fig. 3E). Therefore, our results show that the transfectant DNAs contain *N-ras* genes with a mutation at the second position of codon 13. Recently, we have found that the *N-ras* gene in transfectant DNA of AML 73 also

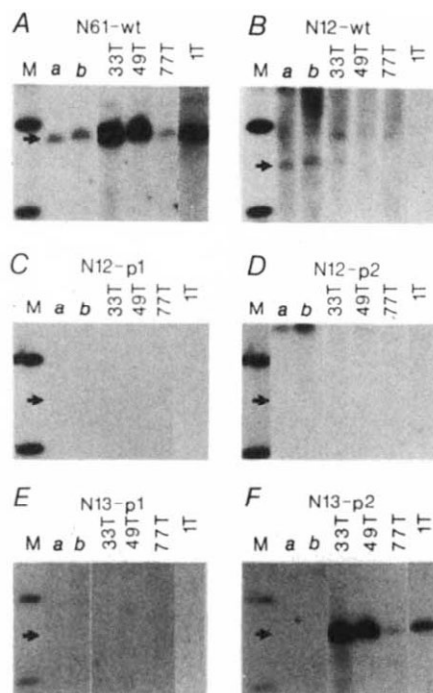


Fig. 3 Hybridization of synthetic oligomer probes to genomic DNA of 3T3 cell transfectants of four different human AMLs. The panels are replicates of each other with DNAs of HL60 (a) and HT1080 (b) as control and the DNAs of the AML transfectants 33T, 49T, 77T and 1T. The panels were hybridized to different oligomer probes: A, N61-wt; B, N12-wt; C, N12-p1; D, N12-p2; E, N13-p1; F, N13-p2; M, fragments of phage λ DNA digested with *Hind*III (4.3 and 2.3 kb). Arrows indicate the position of the N-ras fragment.

Methods. Hybridization was essentially as described by Bos *et al.*¹⁷. Genomic DNA (10 μ g) was digested with *Pst*I and electrophoresed on a 0.5% agarose gel. The gels were denatured in 0.4 M NaOH, 0.8 M NaCl, neutralized in 0.5 M Tris-HCl (pH 7.2), 1.5 M NaCl and dried. The dried gel membranes were hybridized at 50 °C with N61-wt and at 53 °C with the other probes in 5 $\times$ SSPE (SSPE = 10 mM sodium phosphate pH 7.0, 0.18 M NaCl, 1 mM EDTA), 0.3% SDS and 10 μ g ml⁻¹ sonicated *Escherichia coli* DNA. Hybridized gels were washed in 2 $\times$ SSPE, 0.1% SDS at room temperature, in 5 $\times$ SSPE, 0.1% SDS at 53 °C for 15 min and finally in the same solution at either 59 °C (for N61-wt) or 63 °C (for the N12/13 probes) for 5 min. Gel membranes were autoradiographed for 2–4 days using intensifying screens.

Fig. 4 Hybridization of separated N13-p2 oligomer probes to genomic DNAs of the AML transfectants. The panels are replicates of each other, with *Pst*I-digested DNAs of MOLT-4 as control and the DNAs of AML 33T, 49T, 77T and 1T. The panels were hybridized to N13-p2/a (TTCCCAACATCACCTGCTCC; panel A), to N13-p2/b (TTCCCAACAACACCTGCTCC; panel B) and to N13-p2/c (TTCCCAACAGCACCTGCTCC; panel C). Arrows indicate the position of the N-ras fragment; M, phage λ DNA digested with *Hind*III; p, 10 genomic copies of plasmid pAT8.8. This plasmid contains an 8.8-kb fragment of the activated N-ras gene HT1080 (CAA₆₁-AAA; ref. 24).

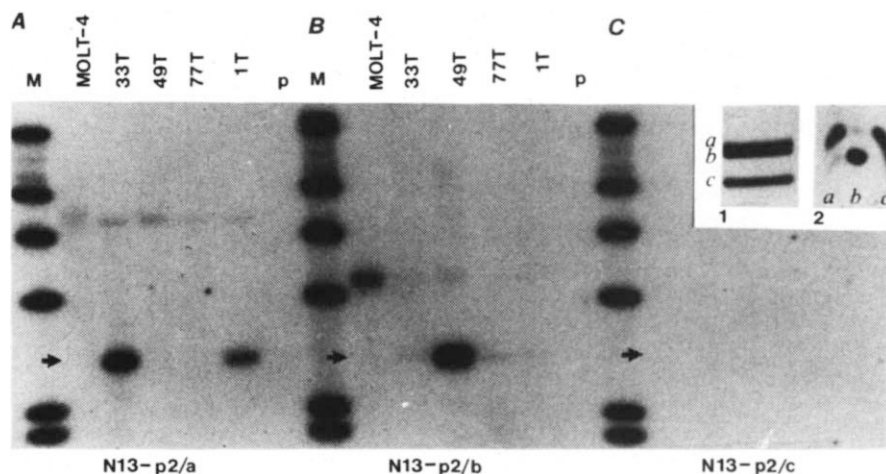
Methods. The three members of oligomers N13-p2 (a, b and c in inserts 1 and 2) were separated on a 20% polyacrylamide gel (mono/bis = 62.5:1) in 0.5 $\times$ TBE (TBE = 90 mM Tris, 90 mM boric acid, 2.5 mM EDTA) (see insert 1). To eliminate contamination, the isolated oligomers were further purified by electrophoresis on cellulose acetate strips in 7 M urea, 5% acetic acid, 5 mM EDTA pH 3.5 at 5,000 V (see insert 2). The fragments were eluted in 1 mM EDTA and used directly for hybridization. The sequence of each oligomer was determined by synthesizing N13-p2 with an end-labelled primer and cold deoxynucleoside triphosphates. This end-labelled probe was separated into the three members of N13-p2 and sequenced according to Maxam and Gilbert²⁶. Gel separation and hybridization were as described in Fig. 2 legend.

hybridizes with the N13-p2 probe (data not shown) and thus contains a mutation at the same position.

Mutation to valine or aspartic acid

The use of mixed oligonucleotide probes allows the detection of the position of a mutation in a codon rather than establishing the precise mutant sequence. To analyse the exact nature of the mutations present in the N-ras gene of the AML transfectants, we hybridized DNAs to the individual oligonucleotide probes corresponding to the three possible mutations at the second position of codon 13. To prepare these individual probes, the labelled oligonucleotides of group N13-p2 were electrophoresed on a polyacrylamide gel (Fig. 4, insert 1), the individual bands excised and the separated oligomers further purified by electrophoresis on cellulose acetate strips (Fig. 4, insert 2).

To identify the separated oligomers, they were sequenced as described in Fig. 4 legend. The slowest-running oligomer (N13-p2/a) on the polyacrylamide gel had the sequence TTCCCAACATCACCTGCTCC and thus identifies a GAT codon 13 (Asp) in a fully matched hybrid. The second oligomer (N13-p2/b) identifies a GTT codon (Val), whereas the third and fastest-running oligomer (N13-p2/c) identifies a GCT codon (Ala). The separated oligonucleotide probes were then hybridized to *Pst*I-digested DNA from four AML transfectants. As shown in Fig. 4A, oligomer N13-p2/a forms a fully matched hybrid with DNA of transfectants from AML 1 and 33, whereas oligomer N13-p2/b forms a fully matched hybrid with DNAs from transfectants of AML 49 and 77 (Fig. 4B). The probe N13-p2/c did not hybridize with any of the transfectants (Fig. 4C) or with any of the separated oligomers, to control MOLT-4 DNA or to 10 genomic copies of the plasmid pAT8.8 (refs 13, 24), which contains the wild-type sequence at codons 12 and 13 (lane p). From these results we conclude that the N-ras gene in transfectants of AML 1 and 33 DNA contains a GAT codon 13, substituting Asp for Gly, whereas the transfectants from AML 49 and 77 contain a GTT codon 13, encoding Val. Recently, we found that AML 73 also hybridized specifically with N13-p2/b and thus contains a Val at position 13 (data not shown). The fact that the transfectant DNAs form fully matched hybrids with different members of the N13-p2 group is further evidence for the specificity of the hybridization, as each DNA now serves as a control for the other. There is no apparent correlation between the classification of the AMLs and the amino-acid substitution: AMLs 1 and 33 (Asp) were classified on the FAB scheme as M4 and M1 respectively, whereas AMLs 49, 73 and 77 (Val) were classified as M5, M2 and M4 respectively.



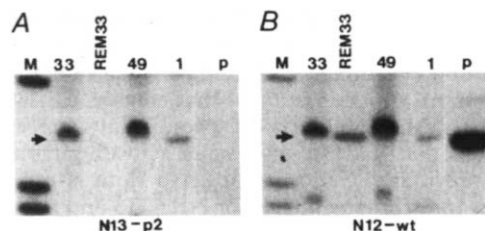


Fig. 5 Hybridization of N13-p2 (A) and N12-wt (B) oligomer probes to genomic DNA of isolated blast cells of AML patients 33, 49 and 1, and to genomic DNA of blood cells from AML patient 33 after remission (REM33). The differences in hybridization signal between the lanes result from loading differences and not from amplification of the *N-ras* gene. Arrows indicate the position of the *N-ras* band. Lane p, 20 genomic copies of the plasmid pAT8.8 containing the wild-type sequence at codons 12 and 13 (refs 13, 24). Gel separation and hybridization were as described in Fig. 3 legend.

Somatic mutation in leukaemic cells

To exclude the possibility that the mutations at codon 13 were acquired during transfection and selection for transformed cells, the DNAs from three leukaemias were analysed. DNA was digested with *Pst*I, electrophoresed on an agarose gel and hybridized *in situ* to the oligonucleotide probes N12-wt and N13-p2. Hybridization of the gel to the mutant-specific N13-p2 oligomer group showed that all three AML DNAs hybridized, but no hybridization was detected to a sample containing 20 copies of a plasmid carrying the wild-type 12 and 13 sequences (Fig. 5A). Thus, the DNAs from these original leukaemic cells also contain the mutation at codon 13, eliminating the possibility that the mutations had arisen as a result of experimental manipulation.

The availability of DNA prepared from the peripheral blood of one patient (no. 33) in remission (REM33) allowed us to investigate whether the codon 13 change was present in normal cells. Figure 5A shows that REM33 DNA did not hybridize with the N13-p2 mutant-specific probes. As a control, we rehybridized the gel with the N12-wt probe and obtained a signal with the normal-sequence probe hybridized to REM33 DNA (Fig. 5B). Therefore, we conclude that this DNA (and thus the normal tissues of the patient) does not contain an altered *N-ras* gene hybridizing with N13-p2, and that the altered *N-ras* gene has arisen by somatic mutation. Hybridization with the normal-sequence probe was also obtained with the three DNAs from leukaemias, which could result from the presence of normal cells in the leukaemic sample. However, the high white blood cell counts of the leukaemic samples ($54\text{--}140 \times 10^9$ cells per l), coupled with the high proportion of cells with blast morphology (95–100%), makes it likely that the hybridization signal with the N12-wt probe is a result of the leukaemic cells containing both a normal and a mutant *N-ras* gene. The presence of amplified copies of the *N-ras* gene in some transfectants suggested that the position 13 mutations might act in concert with an increased copy number of the *N-ras* gene, but examination of the AML DNAs by Southern blotting shows no evidence of *N-ras* gene amplification (data not shown).

Restriction site polymorphism

The *N-ras* 13 mutation in AML33 and AML1 (Gly-Asp) changes the sequence GGTGG to GGTGA, which is the recognition site for the restriction enzyme *Hph*I. This new *Hph*I site will shorten a 550-bp *Pvu*II/*Hph*I fragment by 60 bp (see Fig. 6). We have analysed the original leukaemic DNA of AML33 and transfectant DNAs from AMLs 1 and 33 for the presence of this extra *Hph*I site by Southern blot analysis. To that end we have compared the length of the *Pvu*II/*Hph*I fragments in these DNAs with the lengths of fragments in *N-ras* genes containing the wild-type sequence at and around codon 12 and in a codon

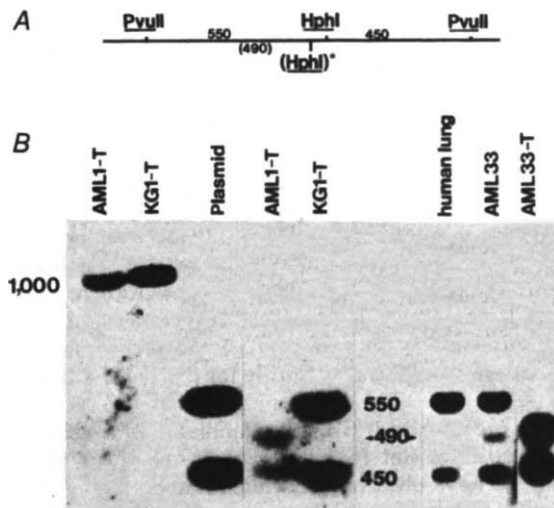


Fig. 6 A, Map of *Pvu*II and *Hph*I restriction enzyme sites in and around the first exon of *N-ras* to show the sizes of fragments generated after digestion of the normal *N-ras* gene and *N-ras* with Asp at codon 13. Restriction enzyme sites are taken from Brown *et al.*¹³, Hall *et al.*²⁴ and A. Hall (personal communication). B, Southern blot analysis to show the altered *Pvu*II/*Hph*I fragments resulting from the presence of a new *Hph*I site generated by the Asp 13 mutation in the original leukaemic DNA from patient AML33 and in transfectant DNAs of AML1 and AML33. This new *Hph*I site results in a *Pvu*II/*Hph*I fragment of 490 bp compared with 550 bp in wild-type DNA (see map).

Methods. DNA was digested either with *Pvu*II or *Pvu*II and *Hph*I, electrophoresed on a 1.4% low gelling temperature agarose gel and transferred to a nitrocellulose filter. The filter was hybridized with the nick-translated 1.0-kb *Pvu*II/*Pvu*II fragment of plasmid pAT7.8 (refs 13, 24) (see map). DNAs: pAT7.8 is a plasmid containing the *Bam*HI/*Eco*RI fragment with the first two exons of *N-ras* cloned from normal DNA¹³, AML33 is the original leukaemic sample, AML33T a tumour derived after co-transfection into NIH 3T3 cells, AML1T is a tumour derived from a focus isolated after transfection of NIH 3T3 cells with AML1 DNA (note that this is a different transfectant from that used in Figs 3, 4 and 5 and contains fewer copies of *N-ras*), KG1-T (a transfectant from KG1 DNA that contains a position 12 mutation; J.W.G.J. and J.L.B., unpublished).

12 mutant (for details see Fig. 6 legend). The digestion of a plasmid, pAT7.8, containing the first and second exons of a normal *N-ras* gene¹³ and of a transfectant, KG1-T, mutated at codon 12 (J.W.G.J. and J.L.B., unpublished results) generates a 550-bp *Pvu*II/*Hph*I fragment and a 450-bp *Hph*I/*Pvu*II fragment when hybridized with a probe of the *Pvu*II/*Pvu*II fragment of pAT7.8 (Fig. 6). However, when DNA from AML33 is digested with *Pvu*II and *Hph*I, a novel 490-bp fragment, as well as the 450- and 550-bp *Hph*I/*Pvu*II fragments, is detected. The presence of both the 550-bp and the novel 490-bp fragment confirms our observation with oligonucleotide probes that this DNA contains *N-ras* genes with both a wild-type codon 13 and an Asp 13 mutation (compare Fig. 5). Southern blotting of a transfectant from AML33 (AML33-T) and a transfectant of AML1 (AML1-T) shows that these contain only the novel 490-bp and the 450-bp *Hph*I/*Pvu*II fragments as expected, because transfectants will contain only the mutated transforming *N-ras* allele. As a new *Hph*I site could not have been generated by a mutation in codon 12, the restriction enzyme analysis confirms the observations with oligonucleotide probes that AML1 and AML33 contain a codon 13 mutation of Gly to Asp.

Discussion

We have found that DNAs isolated from AML contain altered *N-ras* genes. These activated oncogenes have also been detected in three other samples of AML cells from patients^{27–29}; in three AML cell lines, HL60 (ref. 28), Rc2a and KG-1 (ref. 38); and

recently in three more AML samples (D.T. and C.J.M., unpublished results). Why there should be a bias towards activation of *N-ras* genes in these leukaemias is not clear, but it is noteworthy that also in cell lines of acute lymphoblastic leukaemia, *N-ras* normally seems to be the activated *ras* oncogene^{27,30}. *N-ras*, rather than the other *ras* genes, may be critically involved in the control of proliferation and differentiation of cells in the haematopoietic lineage. Alteration of this gene may then lead to the malignant state of the cells. The activation of the same *ras* oncogene in multiple isolates of similar tumours is reminiscent of findings that there is a reproducible pattern of *ras* gene activation in tumours induced by the same carcinogenic agent³¹⁻³³. Therefore, a specific carcinogenic agent may be responsible for the *N-ras* activations in AML.

Unlike previously described examples of *ras* gene activation³⁻¹⁷, we have not found mutations in codons 12 or 61. Instead, we have observed that mutations occur in codon 13, resulting in a replacement of Gly by either Val or Asp. One example of a codon 12 and a codon 61 mutation in AML cells has been noted previously^{17,34} but, as only ~10-20% of malignancies seem to contain *ras* genes with mutations at codon 12 or 61 (for example, see ref. 16), the absence of codon 12 or 61 mutations in the samples we have analysed may result from the comparatively small sample size. The detection of codon 13 mutations in our samples of AML raises the question of why they have not been seen before. If codon 13 changes are restricted to AML, then they may not have been reported previously because only two mutant *N-ras* genes have been characterized from AML^{17,34}. Furthermore, most of our samples were of FAB subtypes M1, M4 and M5, which have not been studied before. A restriction of codon 13 mutations to AML would imply that there is some constraint on the type of *N-ras* mutation in AML. Such a constraint could arise through the mechanism of mutation or through selection for the type of alteration in p21 protein. If codon 13 mutations are found in other malignancies, it is surprising that they have not been reported previously.

Our experience indicates that *N-ras* codon 13 mutants can be relatively inefficient in inducing foci. Although foci were detected in one laboratory with a DNA, AML 1, containing Asp 13 (ref. 38), they were not detected in another laboratory with DNA containing the same mutation, AML 33 (D.T. and C.J.M., unpublished results). This discrepancy has now been resolved by the observation that when the focus assays were kept for longer, foci induced by DNA derived from AML 33 were apparent 19-20 days after transfection but not at the usual time of examination at 15-16 days. However, foci from DNAs con-

taining Val 13 were still not apparent even after leaving the assays for 20 days. Cells cultured from tumours resulting from co-transfection with DNAs containing the Asp 13 mutation also look more transformed than those that contain Val 13 mutants (D.T. and C.J.M., unpublished). These results are consistent with the idea that Val 13, which is an activating mutation not identified previously¹⁸, and perhaps Asp 13 in *N-ras*, are relatively weak transforming alleles for NIH 3T3 and may be more difficult to detect than codon 12 or 61 mutations.

Our observation of multiple cases of mutations at codon 13 suggests that the activation of the *N-ras* gene at this site is an essential step in causing these AMLs. However, it is unlikely that the activation of the *N-ras* oncogene is the only step involved in the genesis of AML, because much evidence favours the argument that changes to more than one gene are involved in neoplastic transformation (see ref. 1 for review). Furthermore, specific chromosome translocations and other aberrations are associated with AML³⁵. These chromosome aberrations occur at sites distinct from the 1cen-1p21 chromosomal site of *N-ras*³⁶ and separate aberrations are found in the different subgroups of AML. For example, a translocation, t(8;21), is found in AML M2 and an inversion of chromosome 16, inv16, in AML M4 with increased eosinophils (see ref. 35 for review). In contrast, we have found activation of *N-ras* in samples of AML from four different subgroups (M1, M2, M4 and M5); thus, the activation of *N-ras* seems to be affecting a process that is essential to all cells in the myeloid lineage, whereas the chromosome aberrations affect genes whose activities are more limited.

For the analysis of the *N-ras* mutations, we have used a set of specific oligonucleotide probes. This assay system is not only powerful in the detection of mutations in transfectant DNAs but also in the detection of specific mutations in tumour DNA. Using this assay system and the *HphI* polymorphism generated by the Asp 13 mutation, we are currently analysing a large number of AML DNAs to gain more precise insight into the occurrence of different *ras* gene mutations in this leukaemia.

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Meteorites may follow a chaotic route to Earth

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It is widely believed that meteorites originate in the asteroid belt, but the precise dynamical mechanism whereby material is transported to Earth has eluded discovery. The observational data for the ordinary chondrites, the most common meteorites, impose severe constraints on any proposed mechanism. The ordinary chondrites are not strongly shocked, their cosmic ray exposure ages are typically <20 Myr, their radiants are concentrated near the antapex of Earth's motion and they show a pronounced 'afternoon excess' (for every meteorite which falls in the morning two fall in the afternoon). Wetherill¹ concluded that these data could only be explained by an "unobserved source" of material with perihelia near 1.0 AU and aphelia near Jupiter. His subsequent, more sophisticated investigations have not changed this basic conclusion. Recently I have shown^{2,3} that there is a large chaotic zone in the phase space near the 3/1 mean motion commensurability with Jupiter and that the chaotic trajectories within this zone have particularly large variations in orbital eccentricity. Since asteroidal debris is quite easily injected into this chaotic zone, it could provide Wetherill's 'unobserved source' if chaotic trajectories which begin at asteroidal eccentricities ($e < 0.2$) reach such large eccentricities that Earth's orbit is crossed ($e > 0.57$)⁴. In this report I present a numerical integration which demonstrates that at least some of these chaotic trajectories do have the properties required to transport meteoritic material from the asteroid belt to Earth. Combined with the Monte Carlo calculations which show that the resulting meteorites are consistent with all the observational constraints, the case for this chaotic route to Earth is fairly strong.

Wetherill^{5,6} has completed an extensive new Monte Carlo study to determine the meteoritic yield from this chaotic zone, with some reasonable assumptions concerning the actual dynamics. He assumes that on a timescale of 10^6 years, chaotic trajectories evolve to become Earth-crossing. During much of this time the trajectories are Mars-crossing. He estimates that there is a 50% chance that the material will become Earth-crossing before Mars removes it from the chaotic zone. (It has been suggested previously⁷ that meteorites could be brought to Earth from the 3/1 resonance with the assistance of Mars. The timescale for this process is, however, longer than the time for collisional disruption of meteorite-sized bodies, and fragments from any larger bodies which have taken this route will not satisfy the orbital constraints for the ordinary chondrites. It is essential, therefore, that trajectories can become Earth-crossing without the assistance of Mars.) Once the fragment becomes Earth-crossing, however, perturbations from Earth remove it from the chaotic zone in 10^4 years, the approximate duration of the Earth-crossing spikes in eccentricity. With this assumed dynamical input, the subsequent evolution is followed with the Monte Carlo method^{8,9}. Wetherill's model includes planetary close encounters, secular resonances, collisional destruction in space and atmospheric ablation. He concludes that major quantities of asteroidal fragments from bodies adjacent to the 3/1 gap can be transferred to Earth. His computed flux is comparable to the observed flux of ordinary chondrites. The afternoon excess of meteorite-sized fragments obtained directly from the chaotic zone is too large, but when the fall-times of these fragments are combined with the fall-times of fragments obtained from larger objects derived earlier from the 3/1 gap, the predicted fall-time ratio agrees with the ordinary chondrite value of 2/3. Similar agreement is found for the other observable parameters.

Greenberg and Chapman¹⁰ have also considered the consequences of this transport mechanism; while they also compute a flux of chondrites consistent with the observed flux, their physical assumptions and methods are quite different from those of Wetherill. The purpose of this letter is not to enter that debate, but to make firm the dynamics on which it is based. If trajectories in the 3/1 chaotic zone do not have the required properties the discussion is moot.

In this article 'chaotic behaviour' refers to deterministic solutions of hamiltonian systems of equations that behave erratically and show sensitive dependence on initial conditions (positive Lyapunov characteristic exponent). On theoretical grounds¹¹, a chaotic zone is generally expected to be associated with each commensurability in a dynamical system, though these chaotic zones are often microscopically small. Using Schubart's numerical averaging procedure¹², Giffen¹³ showed that the 2/1 commensurability in the planar-elliptic restricted three-body problem is accompanied by a sizeable chaotic zone. Scholl and Froeschle^{14,15} extended Giffen's work to the other principal commensurabilities; while they found chaotic behaviour near the 3/1 commensurability, they concluded that the chaotic zone was small and unimportant. In fact, there is a large chaotic zone near the 3/1 commensurability, and trajectories within this zone have the peculiar property that their orbital eccentricity can remain low ($e < 0.1$) for as long as a million years and then suddenly assume very large values ($e > 0.3$)^{2,3}. The long-term evolution of a typical chaotic trajectory has bursts of low eccentricity behaviour followed by bursts of high eccentricity behaviour, each of apparently random duration. Furthermore, when the effects of phase mixing are removed, the boundaries of the 3/1 Kirkwood gap correspond, within the errors of the asteroid orbital elements, to the boundary in the phase space of the chaotic zone. The 3/1 Kirkwood gap can be explained entirely by removal of the highly eccentric chaotic orbits and the also highly eccentric quasiperiodic librators by collisions with Mars and, as reported here, Earth.

These discoveries depended on the development of a new 'mapping' technique for studying the long-term evolution of trajectories near commensurabilities that is approximately 1,000 times faster than all previous methods. This method is approximate (as are the methods used in earlier studies), but the essential features of the picture presented above have been verified by conventional numerical integrations of the exact equations of motion for the planar-elliptic restricted three-body problem³. In particular, the width of the chaotic zone is in perfect agreement with that found using the mapping method. Even at eccentricities as high as 0.4, the trajectories using the mapping and the exact solutions are in good qualitative agreement, even though fourth-order terms in eccentricity are neglected in the disturbing function. Independent investigation has confirmed this qualitative agreement¹⁶.

In the planar-elliptic problem, the eccentricity of chaotic trajectories which begin at asteroidal eccentricities ($e < 0.2$) seems to be limited to values < 0.5 ; this is large enough for the trajectories to be Mars-crossing but not Earth-crossing. However, in the three-dimensional elliptic problem, chaotic trajectories computed with a mapping reach much larger eccentricities, large enough to be Earth-crossing ($e > 0.57$). Now the approximations used to derive the mapping are certainly invalid at such large eccentricities, but the success of the planar mapping at eccentricities as large as 0.4 encourages one to take these high eccentricities seriously. The actual eccentricity limit can be determined only by numerical integration of the exact equations of motion. Such integrations are time-consuming, but the answer is vital to our understanding of the origin of meteorites.

One approach to this problem is simply to integrate a chaotic trajectory until it reaches Earth-crossing eccentricities. Experience with the mapping, however, indicates that this may take several million years. Furthermore, not all chaotic trajectories are equivalent; there is an integral which divides the chaotic zone into a one-parameter family of chaotic zones. Some

of these may have Earth-crossing trajectories and others not; we do not accurately know the extent of the eccentricity variations for any chaotic trajectory. It might then be necessary to integrate several chaotic trajectories for several million years to find the Earth-crossing route. This approach is not practical without a dedicated computer. Instead, I have sought, by trial and error, trajectories which begin near that point in the phase space where transitions from low eccentricity behaviour to high eccentricity behaviour occur, with as much guidance from the mapping as I have been able to glean. (While the mapping does give the correct qualitative behaviour, the correspondence is not precise enough to directly determine initial conditions.)

The numerical integrations include the Sun, the four major planets and a test body. Pluto is clearly not important because of its small mass. The inner planets are included with the Sun. I am attempting to show that the long-term evolution of a chaotic trajectory without close planetary encounters will bring material within the influence of Earth's gravitational field. It seems fair to ignore Earth until this is the case. The neglect of Mars perturbations is clearly more serious. I argue that Mars substantially affects the solution only during close encounters and that these close encounters may be taken into account after the fact in a statistical manner, much as Wetherill⁶ has done. (His approach may not be entirely satisfactory. As mentioned previously, there is a family of chaotic zones, not just one. Minor encounters with Mars will be sufficient to cause a shift from one member of this family to another without removing the trajectory entirely from the chaotic zone. Several such shifts may occur before Earth is reached. Nevertheless, such trajectories would essentially be injected directly into Earth's control. Thus this synergism between Mars perturbations and chaotic dynamics is qualitatively different from previously proposed mechanisms⁷ that rely on Mars perturbations to assist in the transfer of material from the Kirkwood gaps.)

The integrations were performed in equatorial rectangular coordinates referred to the mean equinox and equator 1950.0. The origin was the barycentre of the Sun and four inner planets. The unit of time was 40 mean solar days; the origin of time was taken to be 1982 August 19.0 (JD 2,445,200.5). The extrapolation algorithm of Bulirsch and Stoer¹⁷ was used to perform the integrations. The relative accuracy per integration step was chosen to be 10^{-11} ; the variable integration step was of the order of 1 yr. The twelfth-order Cowell method used by Cohen *et al.*¹⁸ in their 1-Myr integration is less satisfactory when the eccentricities are large as in the integrations reported here. The initial conditions for the major planets were obtained from those used by Cohen *et al.* by numerical integration from their origin of time (JD 2,430,000.5) to mine. Their initial conditions for the major planets were in turn taken from the work of Eckert *et al.*¹⁹, where the equations of motion can be found. No effort has been made to correct the initial conditions to properly account for my removal of Pluto. Because of the large Pluto mass used by Eckert *et al.* this introduces a relative discrepancy

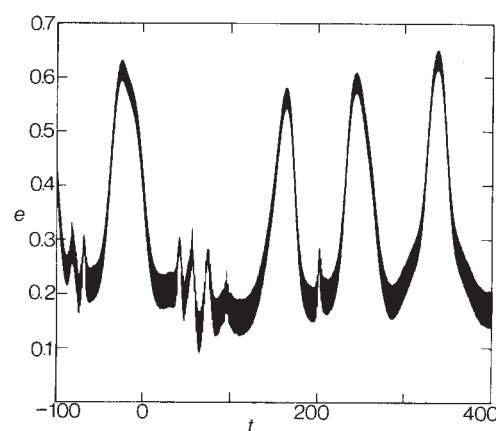


Fig. 1 The eccentricity (e) as a function of time (t , in millennia) plotted for the chaotic trajectory, which at times is deep in the asteroid belt (when $e < 0.15$) and at other times is strongly Earth-crossing ($e > 0.6$). The time origin is 1982 August 19.0.

of one part in a million in the Cartesian coordinates just in the integration from their origin of time to mine. (I have verified, however, that when Pluto is included the program satisfactorily reproduces the tables of Eckert *et al.*¹⁹.) The accuracy of these numerical integrations was tested directly by integrating the planets forward with one test meteoroid 170,000 years, and then integrating them back to the time origin with a different meteoroid on an independent trajectory. Since the time step chosen by the integrator is mainly determined by the eccentricity of the meteoroid, these two integrations are completely independent. The initial conditions of the major planets were reproduced to one part in 10^4 . The skeleton of the integration is thus adequately represented.

It is well known that chaotic trajectories are difficult to follow precisely. In a chaotic zone neighbouring trajectories separate exponentially with time, and consequently so do round-off errors. Fortunately, the rate of this exponential separation is rather small for the 3/1 chaotic zone³; nevertheless, even a small exponential growth of errors is unbeatable. Now it has been argued²⁰ that even though any particular trajectory cannot be followed precisely, the qualitative behaviour of the trajectory will be correct if reasonable care is taken and furthermore that a true trajectory can be found which shadows the computed trajectory. This is not as wild a claim as it may seem, for it has been proven for the special class of Anosov dynamical systems. It is probably not strictly true for Hamiltonian systems in general, but most people who study chaotic behaviour in dynamical systems believe that it is practically true. The actual rate of separation of nearby trajectories in the 3/1 chaotic zone allows strict confidence to be placed in segments of 80,000 years³. Over longer times we may only hope that some true trajectory shadows

Table 1 Equatorial frame coordinates and velocities at JD 62043200.5

	x (AU)	y (AU)	z (AU)
Meteoroid	$0.262137541577 \times 10^1$	$-0.949476934922 \times 10^0$	$-0.179096284774 \times 10^0$
Jupiter	$-0.351023142831 \times 10^1$	$0.339348310152 \times 10^1$	$0.151726945011 \times 10^1$
Saturn	$0.777797463489 \times 10^0$	$-0.824734443595 \times 10^1$	$-0.369212909944 \times 10^1$
Uranus	$-0.748876946495 \times 10^1$	$-0.165985490544 \times 10^2$	$-0.644552105739 \times 10^1$
Neptune	$0.287412144601 \times 10^2$	$0.842938531215 \times 10^1$	$0.260621846977 \times 10^1$
	$\dot{x}$ (AU/40 days)	$\dot{y}$ (AU/40 days)	$\dot{z}$ (AU/40 days)
Meteoroid	$0.311286716224 \times 10^0$	$0.216759866523 \times 10^0$	$0.772871903870 \times 10^{-1}$
Jupiter	$-0.212645617591 \times 10^0$	$-0.206291875136 \times 10^0$	$-0.807809459066 \times 10^{-1}$
Saturn	$0.232711603323 \times 10^0$	$0.234902479708 \times 10^{-1}$	$0.286504328835 \times 10^{-2}$
Uranus	$0.145063206654 \times 10^0$	$-0.519858201645 \times 10^{-1}$	$-0.249322339022 \times 10^{-1}$
Neptune	$-0.382851184016 \times 10^{-1}$	$0.109983656044 \times 10^0$	$0.465724813196 \times 10^{-1}$

the computed trajectory. In any case 80,000 years is long enough to cover the most interesting behaviour.

I have made five numerical integrations, each spanning ~500,000 years. The initial conditions were chosen in the chaotic zone as determined by the three-dimensional mapping. The initial eccentricity for the first four was 0.15, the mean eccentricity of the asteroids; for three of these the resulting peak in eccentricity was between 0.5 and 0.54, insufficient for Earth-crossing. Earth's maximum aphelion occurs at 1.07 AU (ref. 21); for semimajor axes near the 3/1 commensurability ($a \approx 2.5$ AU) an eccentricity of 0.57 is required to just graze this distance. The results of the first three integrations were encouraging but not sufficient. The fourth integration, however, reached an eccentricity of 0.564 after 170,000 years. Its initial conditions (JD 2,445,200.5) were: semimajor axis $a = 0.4816a_J$, eccentricity $e = 0.15$, inclination $i = 5^\circ$, mean anomaly $M = 180^\circ + 3M_J$, longitude of perihelion $\varpi = \varpi_J$ and longitude of ascending node $\Omega = \varpi_J$. These elements are heliocentric osculating elements relative to the equinox and ecliptic 1950.0, and the subscript J refers to Jupiter. These initial conditions are on the representative plane³. This trajectory came so close to Earth-crossing (0.01 AU) that it appeared possible to change the coordinates enough to make it Earth-crossing and still remain in the chaotic zone. The first attempt to do this was, however, unsuccessful. The resulting trajectory was clearly quasiperiodic in nature. With a somewhat smaller increase in eccentricity, however, the route to Earth was found.

The initial conditions for this chaotic trajectory are given in Table 1. The trajectory was integrated both forward and backward in time from that point. The eccentricity as a function of time (in millennia) is shown in Fig. 1; the peak in eccentricity occurs near $t = 340,000$ yr with the value $e_{\max} = 0.652$; the minimum perihelion distance is $q_{\min} = 0.861$ AU, which is strongly Earth-crossing. The peak near $t = -20,000$ yr has $e_{\max} = 0.632$ and $q_{\min} = 0.910$ AU. The minimum in eccentricity occurs near $t = 60,000$ yr during a short burst of low eccentricity behaviour with the value $e_{\min} = 0.089$; this value is lower than the mean eccentricity of the asteroids. The semimajor axis remains between 2.47 AU and 2.53 AU as expected. The inclination to the ecliptic varies between 1° and 8° in a way that is not obviously correlated with the eccentricity. The behaviour of the resonant argument $\sigma = l + 2\varpi - 3l_J$, where l is the mean longitude, is very complicated. During the eccentricity spikes it librates about a slowly varying centre which makes one revolution during the spike; during the low eccentricity bursts it circulates. The behaviour of the semimajor axis is correlated with the resonant argument: during the eccentricity spikes the semimajor axis oscillates across its full range of variation, but during the low eccentricity bursts it alternately oscillates on one side or the other of the centre of that range. This behaviour is familiar from the mapping studies, and consistent with the qualitative picture of these bursts presented previously³. The maximum Lyapunov exponent for this chaotic trajectory is $\lambda \approx 10^{-3.8} \text{ yr}^{-1}$.

The peak in eccentricity that occurs near 170,000 years, near our starting point, has $e_{\max} = 0.583$ and $q_{\min} = 1.034$ AU. Since $q_{\min}$ is less than Earth's maximum aphelion of 1.07 AU I was initially satisfied that this trajectory was indeed Earth-crossing; on closer examination this turned out not to be the case. I first checked that the argument of perihelion (the angle between the longitude of the ascending node and the longitude of perihelion) was satisfactory. It turns out to be correlated with the peaks in eccentricity, and to be ideally placed for Earth-crossing (near zero). If it had been near 90° when the eccentricity was large, then Earth's orbit would not intersect the meteoroid's orbit—the meteoroid's orbit would have crossed the ecliptic near Mars. However, a more subtle avoidance mechanism was operative. During the peaks in eccentricity the longitude of perihelion aligns with Jupiter's longitude of perihelion. Although it is not commonly known, Earth's perihelion is also correlated with Jupiter's perihelion. Earth's maximum aphelion distance always occurs when the perihelia of Earth and Jupiter are aligned. Thus,

if the meteoroid and Earth are both near their maximum eccentricities, their orbits are aligned so that no crossing occurs. It is a simple matter to calculate from Brouwer and van Woerkom's solution²² of the secular motions of the planets that when Earth's perihelion is anti-aligned with Jupiter's perihelion, Earth's maximum aphelion is only 1.031 AU. During this peak the meteoroid came quite close to Earth, but "An inch is good as a mile". Nevertheless, the other peaks in eccentricity are strongly Earth-crossing and no avoidance mechanism is operative.

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Poorly graphitized carbon as a new cosmothermometer for primitive extraterrestrial materials

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The presence of carbon in primitive extraterrestrial materials has long been considered a useful indicator of prevailing geochemical conditions early in the formation of the Solar System. A recent addition to the suite of primitive materials available for study by cosmochemists includes particles collected from the stratosphere called chondritic porous (CP) aggregates¹. Carbon-rich CP aggregates are less abundant in stratospheric collections and contain many low-temperature phases (such as layer silicates) as minor components^{2,3}. We describe here the nature of the most abundant carbon phase in a carbon-rich CP aggregate (sample no. W7029*A) collected from the stratosphere as part of the Johnson Space Center (JSC) Cosmic Dust Program⁴. By comparison with experimental and terrestrial studies of poorly graphitized carbon (PGC), we show that the graphitization temperature, or the degree of ordering in the PGC, may provide a useful cosmothermometer for primitive extraterrestrial materials.

A detailed analytical electron microscope (AEM) investigation of CP aggregate W7029*A has established that parts of this aggregate experienced nominal flash-heating no higher than

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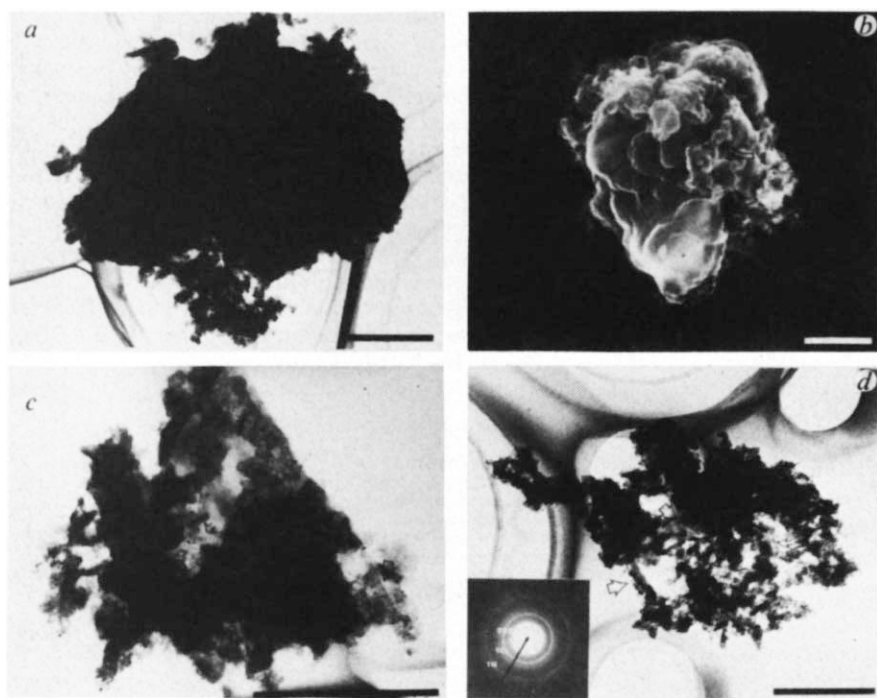


Fig. 1 Examples of carbon particles in CP aggregate W7029*A. *a*, TEM image of clumped carbon particle with granular areas along the margin. *b*, SEM image of the same carbon particle as *a*, but tilted to show individual plates and granular strings (arrowed). *c*, TEM image of granular particle showing two-dimensional fibrous domains (arrowed). *d*, TEM image of an open, fluffy granular cluster with three small sheets (arrowed) and its typical SAED pattern of diffuse rings (inset). Scale bars, 0.5 μm .

300–400 °C during atmospheric entry and that terrestrial contamination of the aggregate was not significant³. The fluffy morphology and chondritic bulk composition of CP aggregate W7029*A, as well as unique mineralogy, are reliable indicators of its extraterrestrial origin^{2,3}. A modal analysis of 224 grains from this CP aggregate shows that ~45% are carbon-rich phases and ~30% are low-temperature phases (such as layer silicates, goethite and sulphides). Further details on the mineralogy of minor phases in CP aggregate W7029*A, as well as collection, curation and processing procedures at the JSC Curatorial Facility are given elsewhere (refs 3, 5 and refs therein). Data on CP aggregate W7029*A were obtained with a JEOL 100CX AEM operating at 100 kV and equipped with a Princeton Gamma Tech System IV energy dispersive spectrometer (EDS). Electron energy loss spectra (EELS) were collected with a Gatan spectrometer attached to a Philips EM 420 AEM operating at 120 kV. The interlayer spacings obtained from calibrated electron diffraction patterns and listed in Table 1 for CP aggregate W7029*A have a relative error of 1%.

Carbon-rich particles in CP aggregate W7029*A form separate clump-like masses. (EDS analyses of 65 clumped masses are consistent with materials in which elements with atomic number >10 are absent. In addition, electron energy loss spectroscopy of selected clumped masses confirmed the presence of carbon only.) Carbonaceous material with similar morphologies have been described in two CP aggregates containing silicate and

magnetite platelet grains⁶. Figure 1*a, b* shows transmission and scanning electron microscope (TEM and SEM) images of a typical carbon particle in CP aggregate W7029*A. The clumps of carbonaceous material range in size from 0.2 × 0.3 μm to 1.5 × 2.0 μm . These clumped masses appear to consist of stacked sheets with lobate circumferences and may have a ragged topography (Fig. 1*b*). Individual sheets may be decorated by fine-scale (0.01–0.05- μm diameter) granular carbonaceous material. These granules may coalesce to form decorative strings around and up on individual sheets, or may form larger granular clusters with an open, fluffy texture (Fig. 1*c, d*). Within these granular clusters, small sheets are occasionally observed (see Fig. 1*d*). TEM images of the clumped masses show two-dimensional fibrous domains (100–1,400 Å long and 45–60 Å wide), open, randomly oriented loops and subcircular structures ~150–500 Å in diameter. Selected area electron diffraction (SAED) patterns of the granular clusters from CP aggregate W7029*A show broad, diffuse rings (inset, Fig. 1*d*) and have 002, 10 $\bar{1}$ and 11 $\bar{1}$ interlayer spacings consistent with PGC⁷. In addition, the range and type of morphologies observed for the carbon phases in CP aggregate W7029*A are similar to those observed for PGC formed by carbonization, that is the removal of oxygen and nitrogen, and subsequent graphitization of hydrocarbons^{8,9}.

Other primitive extraterrestrial materials, particularly the carbonaceous chondrites, contain variable amounts of carbon

Table 1 Interlayer spacing, dimensions of the graphite component and fraction of ordered layers in PGC in three carbonaceous chondrites and CP aggregate W7029*A

	CP aggregate W7029*A	Carbonaceous chondrites		
		Orgueil (CI)	Cold Bokkeveld (CM)	Allende (CV)
d_{002} spacing (range) (Å)	3.50 (3.47–3.53)	3.8 (3.5–4.1)	3.6 (3.4–3.9)	3.47 (3.39–3.5)
L_a (Å)	<100–1,400	60–200	100–420	190–270
Fibres				150–1,000*
L_c (Å)	45–60	20–40	35–140	60–100
Polygonal and subcircular structures (Å)	150–500	2,000–3,500	1,500–2,000	100–150*
Fraction of ordered layers (p)	0	0	26	35

Meteorite data are from refs 7, 10* and CP aggregate W7029*A data from the present study. The number of samples for Orgueil, Cold Bokkeveld meteorites and CP aggregate W7029*A is one; for the Allende meteorite five samples are used. In each sample, a larger number of individual particles have been studied (for example, 65 in the aggregate). Note that the true length of fibrous crystallites (L_a) may be greater because lattice fringes can only be observed if the graphite basal reflection (d_{002}) is parallel to the incident electron beam. L_c , the width (= thickness) of fibrous crystallites.

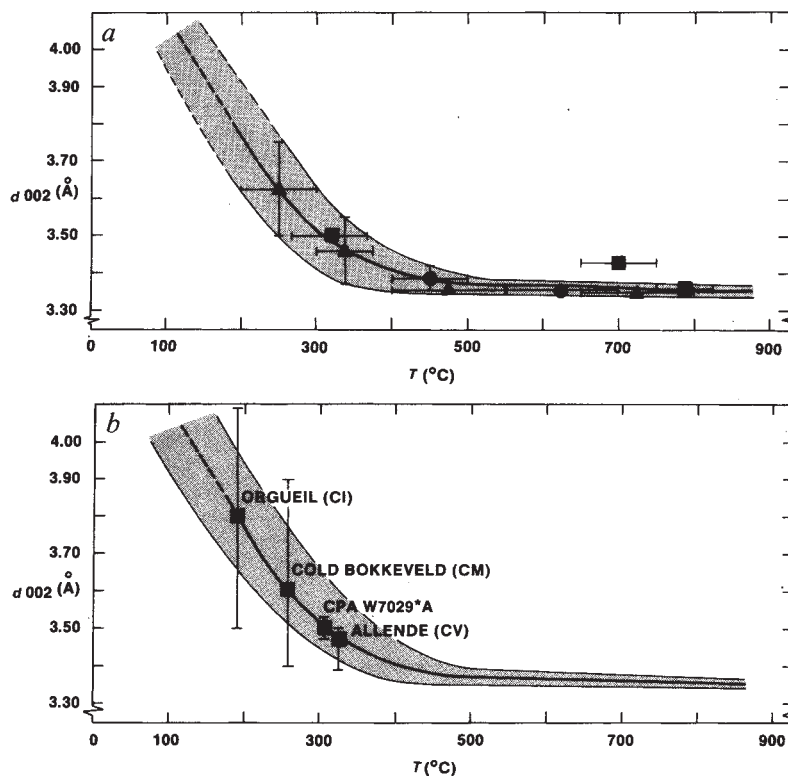


Fig. 2 *a*, Average d_{002} interlayer spacing ($\text{\AA}$) in naturally occurring poorly graphitized carbon in terrestrial metamorphic rocks plotted against temperature ($^{\circ}\text{C}$). The shaded area denotes uncertainty in the data. Sources: $\bullet$, ref. 11; $\blacksquare$, ref. 13; $\blacktriangle$, ref. 14. *b*, d_{002} interlayer spacing in PGC from carbonaceous chondrites and from CP aggregate W7029*A plotted on the curve for PGC in terrestrial metamorphic rocks.

phases. AEM studies of acid residues of the Allende CV meteorite show that carbon phases form as tangled, fibrous (graphitic) crystallites, or aggregates of crystallites, in a featureless (possibly amorphous) matrix¹⁰. Subcircular to polygonal concentric structures with the graphite basal plane spacing were also observed¹⁰. SAED patterns from carbonaceous material in the Orgueil (CI), Cold Bokkeveld (CM) and Allende (CV) meteorites have been obtained by Lumpkin⁷. These SAED patterns typically show broad rings corresponding to the 002, 101 and 111 reflections of turbostratic carbon, or PGC.

The average d_{002} interlayer spacings for each of these primitive materials and for CP aggregate W7029*A are listed in Table 1. For the Orgueil, Cold Bokkeveld and Allende meteorites the size of fibrous crystallites increases uniformly with decrease in average d_{002} interlayer spacing (Table 1). In addition, the diameter of subcircular to polygonal concentric structures decreases from the Orgueil to Cold Bokkeveld to Allende meteorite. In Table 1, all four classes of primitive material show distinct signatures for the PGC. Our compilation of data suggests that a parameter related to the environment of formation may be responsible for the variation in d_{002} spacing, size and morphology of PGC in all four primitive extraterrestrial materials.

There exists a large body of data on the processes of carbonization and graphitization^{8,9}, in both experimental conditions and within natural systems¹¹. These data provide further insight into the probable formation conditions for PGC in CP aggregate W7029*A and selected carbonaceous chondritic meteorites. The d_{002} interlayer spacing is a characteristic structural feature of poorly graphitized carbons which can range from values of 4.8 to 3.44 $\text{\AA}$ (the average interlayer spacing of extensive, randomly stacked graphitic layers) to 3.354 $\text{\AA}$, the value typical of fully ordered hexagonal graphite⁸. Experimental work has shown that this variation in d_{002} interlayer spacing is temperature-dependent. Thus, with heat-treatment temperature the d_{002} interlayer spacing steadily decreases from 4.8 to 3.44 $\text{\AA}$ (ref. 8). The sizes of tangled graphitic fibres are directly related to the formation temperature of poorly graphitized carbon⁹ and increase with increased heat-treatment temperature and/or time. These experiments^{8,9} are of short duration and use pure starting materials. In all experiments, the extent of graphitization of carbon compounds is strongly influenced by the presence of a catalyst¹².

A similar temperature-dependency for d_{002} interlayer spacing PGC can be observed in natural terrestrial environments. Graphitization in terrestrial rocks may occur over long periods of time (millions of years) (long duration of heat-treatment) and may also be influenced by an active fluid phase, lithostatic pressure or the presence of catalytic minerals (such as silicates and carbonates)¹¹. Owing to these influences, in general, the temperature recorded by graphitization of PGC in a metamorphic rock¹¹ will be lower than that determined by experiments with pure starting materials⁸.

Both X-ray diffraction and AEM have been used to study graphitization in terrestrial metamorphic rocks^{11,13-15}. Using data collected with both techniques, we have plotted (Fig. 2*a*) the average d_{002} interlayer spacings for PGC from a suite of metamorphic rocks against their peak metamorphic temperatures. In all cases, peak metamorphic temperatures used in Fig. 2*a* are those given by the original authors^{11,14} except for the sequence described by French¹³. For these data points, we estimated peak metamorphic temperatures using recently published petrological data for these contact-metamorphosed Precambrian iron formations¹⁶⁻¹⁹. The ranges of d_{002} interlayer spacings and peak metamorphic temperatures for each data point are represented by vertical and horizontal error bars, respectively, in Fig. 2*a*. As we show, the average d_{002} interlayer spacing for PGCs in terrestrial metamorphic rocks decreases with increased peak metamorphic temperature up to $\sim 450^{\circ}\text{C}$. Above this temperature the carbon phase may become fully ordered graphite.

The mean apparent interlayer spacing (d_{002}) can be related to the degree of order (degree of graphitization), or the fraction of sp^2 carbon layers in PGC. For synthesized PGCs, an expression relating the mean apparent interlayer spacing and the fraction, p , of ordered layers may be written as $d(002) = 3.44 - 0.086(1 - p^2)$ (ref. 20). Using this equation, we have listed in Table 2 the calculated degree of order for each natural occurrence used to construct Fig. 2*a*. This tabulation indicates that the degree of order determined from average d_{002} values does not vary uniformly with estimated peak metamorphic temperatures. However, we can understand this variation in degree of order for terrestrial rocks if we consider the duration of peak metamorphic heating as well as the peak metamorphic

Table 2 Interlayer spacing and estimated peak metamorphic temperature for PGC from terrestrial metamorphic rocks

d_{002} spacing (Å)	Peak metamorphic temperature (°C)	No. of observations	Degree of order
Poorly graphitized carbon			
3.75–3.50	200–300 (14)	27 (14)	0
3.55–3.37	300–375 (14, 23)		0–57
3.36–3.35	400–500 (14, 23)		74–80
3.50	3.20 ± 50 (16–19)	8 (13)	0
3.43	650–750 (16–19)		6
3.42–3.36	400–500 (11)	8 (11)	12–74
Fully ordered graphite			
3.35	600–800 (14, 23)	8 (14)	100
3.36	750–825 (16–19)	8 (13)	100
3.36	500–700 (11)	8 (11)	100

The numbers in parentheses refer to the source of data. Data points from refs 11, 14 are regionally metamorphosed rocks; data points from ref. 13 are for contact metamorphic rocks. References 16–19 and 23 are used to estimate peak metamorphic temperatures for contact metamorphic rocks in ref. 13 and for regionally metamorphic rocks in ref. 14.

temperature. For example, graphitization takes place in contact metamorphic rocks at ~1,000 °C. However, for regionally metamorphosed rocks a comparable degree of graphite order occurs at ~600 °C if the duration of metamorphic heating is at least 1,000 yr (ref. 11). For metamorphic rocks at lower temperatures, kinetic factors tend to dominate the graphitization process and the range of values for the d_{002} interlayer spacing may reflect a random statistical effect of non-uniform degrees of order within a given sample. Thus, it is appropriate to use the average d_{002} interlayer spacing for individual samples to derive graphitization temperature, or degree of order.

The curve in Fig. 2a shows the relationship between the average d_{002} interlayer spacing and peak metamorphic temperature for PGC in terrestrial metamorphic rocks. In Fig. 2b, the values of the d_{002} interlayer spacing for selected primitive extraterrestrial materials are superimposed on this curve for terrestrial occurrences. We then estimate graphitization temperatures for the carbonaceous chondrites at ~190 °C (Orgueil), ~260 °C (Cold Bokkeveld) and ~335 °C (Allende) and for CP aggregate W7029*A at ~315 °C. The inverse relationship between sizes of fibrous crystallites and diameters of subcircular to polygonal concentric structures⁹ in the carbonaceous chondrites (Table 1) is also compatible with higher temperatures for Allende compared with the Orgueil meteorite.

Graphitization temperatures derived from Fig. 2b are higher than the calculated accretion temperatures for carbonaceous chondrites: <42–87 °C for C1 and CM chondrites, <127 °C for CV chondrites²¹. Graphitization in these meteorites may be reasonably related to post-accretion processes, as graphitization is an irreversible process⁸ and the temperatures obtained from Fig. 2b are probably minimum estimates. The curve in Fig. 2a probably averages the effects of all parameters which influence graphitization of poorly graphitized carbons on a geological timescale. The presence of active catalytic phases will lower the graphitization temperature¹¹. However, unless catalytic activity in these primitive extraterrestrial materials was more pronounced than in the terrestrial occurrences used for Fig. 2a, the curve in Fig. 2b represents graphitization temperatures for PGCs in these extraterrestrial materials. If catalytic activity in these meteorites and CP aggregate W7029*A was indeed more dominant than is assumed for the curve in Fig. 2b all data points would shift towards lower temperatures.

We have shown that graphitization temperatures in terrestrial rocks may be a useful guide to the interpretation of graphitized carbon occurrences in extraterrestrial materials. The degree of graphitization in a suite of primitive extraterrestrial materials follows a simple trend which may be related to the duration and temperature of peak metamorphic heating during formation. Graphitization temperatures recorded in this suite of extraterres-

trial materials and derived from terrestrial trends are consistent with metamorphic temperatures experienced after condensation of the solar nebula but early in the history of the Solar System. Further investigation of graphitization in extraterrestrial materials may provide useful constraints on the formation of parent bodies for primitive chondrites and CP aggregates²².

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Radial distribution studies of non-framework aluminium species formed during dehydroxylation of zeolite HY

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The use of stabilized zeolite Y as a cracking catalyst for converting petroleum into gasoline, fuel oil and petrochemical feeds is widespread¹. During the stabilization process, when the ammonium-exchanged form of zeolite Y is heated to high temperatures, aluminium is extracted from the zeolite framework^{1–3}. The nature of this non-framework aluminium has been of considerable interest because of its possible influence on the zeolite acidity and thermal stability. Indirect information on the nature of the non-framework Al (NFA) has been obtained from infrared spectroscopy, X-ray diffraction, absorption and nuclear magnetic resonance (NMR) studies. To obtain further knowledge of the NFA species, we have now performed radial electron distribution studies of H–Y and H–Y dehydroxylated in vacuum and steam. These studies suggest that the non-framework Al species probably contain edge-shared octahedral $\text{Al}_m(\text{O},\text{OH})_n$ chains similar to those found in $\text{NaAlSi}_2\text{O}_6$ (jadeite), $\text{CaAl}_2(\text{OH})_4\text{SiO}_4$ (chantalite) and $\text{Al}_2(\text{OH})_2\text{TeO}_3\text{SO}_4$.

The loss of Al from the framework and the development of octahedral Al is evidenced by (1) infrared spectroscopy from

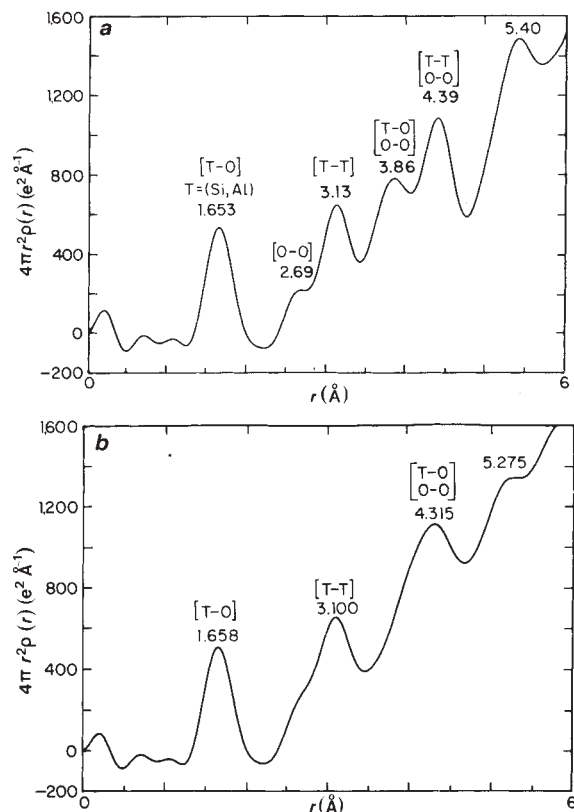


Fig. 1 Experimental RDFs for HY (a) and HY-DH (b). Both in vacuum; temperatures 300 °C (a) and 650 °C (b).

shifts in the infrared (Si, Al)–oxygen (T–O) stretching frequencies^{4–9}; (2) X-ray fluorescence spectroscopy from shifts of the SiK β and AlK β lines^{10–13}; (3) X-ray diffraction from the decrease in cell dimensions^{5,7,8,14}; (4) X-ray structure analyses of dehydroxylated Y¹⁴; (5) ²⁹Si-NMR from increases in the Si/Al ratios^{15–17}; and (6) ²⁷Al-NMR from observation of spectra arising from octahedral Al species (refs 18–20 and P. A. Jacobs *et al.*, in preparation). Infrared spectra in the OH region frequently show weak bands which appear after dehydroxylation. These bands are generally believed to result from OH groups associated with either the NFA species²¹ or vacancies created during the removal of Al^{5,22,23}. There has been no unequivocal identification of these bands, but the species Al(OH)²⁺, Al(OH)₂⁺, AlO⁺, [Al₂O₂OH]⁺, [Al₂O]⁴⁺ and Al(OH)₃ (refs 11, 14, 21, 24–29) have been proposed. Recently, ²⁷Al-NMR studies have indicated that the NFA species in zeolite Y contain octahedrally coordinated Al and are located in large cavities after deammoniation at 300 °C and are distributed in both the large cavities and the sodalite cages after treatment at 500 °C²⁰. A distinction between low-condensed mobile NFA species formed at 300–500 °C under shallow-bed conditions and higher-condensed, immobile species formed at 500 °C under deep bed or moist conditions was made by Freude *et al.*¹⁹ and Raatz *et al.*¹³.

One sample of Na-Y for use in vacuum dehydroxylation was exchanged eight times with 1 M NH₄Cl to yield NH₄-Y-containing 0.99% Na (dry basis). Activation was carried out in a U-shaped quartz cell containing two 5-cm² frits. NH₄-Y (5 g) was heated in flowing O₂ (5 °C min⁻¹) from 25° to 300 °C to eliminate all hydrocarbons. The sample was then evacuated and held for 15 h at 300 °C to obtain H-Y. The quartz cell was opened in a glove box where 1 g of zeolite was transferred to a controlled atmosphere cell used to collect X-ray intensity data. The zeolite powder was pressed into a rectangular cavity in the inside of the back of the X-ray cell which was filled with Ar before removal from the glove box. Vacuum-dehydroxylated Y (HY-DHV) was prepared by transferring the H-Y used for data collection back to the U-tube, heating the sample to 650 °C for

Table 1 RDF spectra for non-framework Al species in HY-DH

<i>d</i> (Å)		
HY-DHV	HY-DHSt	Assignment
—	1.86	Al–O _i
—	2.10	Al–O _{br}
2.87	2.91	Al–Al, O–O
3.39	3.35	Al–O
4.12	4.17	Al–O
4.82	4.84	Al–Al, O–O, Al–O
5.79	—	Al–O, O–O

6 h in a 10⁻⁵ torr vacuum. To minimize possible de-alumination by escaping NH₃ and H₂O, care was taken to heat the sample in a shallow-bed (1 mm thick) configuration and to increase the temperature slowly from 25 to 650 °C under vacuum. The sample was then retransferred to the X-ray sample holder in the glove box. A further sample of NH₄-Y containing 2.2% Na was prepared for steam dehydroxylation. NH₄-Y was heated under 100% H₂O at 600 °C, re-exchanged with NH₄Cl and reheated in 100% H₂O at 890 °C to give steam-dehydroxylated Y (HY-DHSt)³⁰.

The X-ray diffraction patterns were recorded with a stabilized Philips diffractometer using a 2,400-W tube with molybdenum anode (λ (MoK α) = 0.710 Å) using a technique described earlier³¹. The radial distribution functions (RDFs) were calculated by Fourier transformation of the scaled intensities as described previously³².

The calculations of the RDFs of the Al₃O₁₄ clusters were made using the computer program RADMOR developed by D'Antonio and Konnert³³ after modification to run on a VAXS 11/780 computer. The program is described in detail in ref. 33 but we include above a brief description of the quantities calculated and parameters used in our calculations.

RADMOR calculates the following expression:

$$4\pi\rho(r) = \frac{1}{r} \sum_{i=1}^n \sum_{j=1}^m \frac{w_i w_j \bar{f}_i \bar{f}_j \exp[-(r-r_{ij})^2/2\sigma_{ij}^2(r)] \Delta r}{r_{ij} \sigma_{ij}(r) \sqrt{2\pi}}$$

where $4\pi\rho(r)$ is the probability, weighted by the scattering power of the atomic pair ij , ($f_i f_j$), of finding atoms j in a shell of thickness Δr and radius r_{ij} from origin atoms i . w_i is the occupancy of atom i , $f_i = f_i(s=0)/[f^2(s=0)]^{1/2}$ where $s = 4\pi \sin \theta/\lambda$ —is the reduced, scattering angle independent, scattering factor for atom i , r_{ij} is the distance between atoms i and j and $\sigma_{ij}(r)$ includes both the r.m.s. displacement along r_{ij} and positional disorder. The first sum is carried out over the n atoms in the asymmetric unit for a crystalline material. The second sum is carried out over the m atoms in the structure that are separated from an atom in the asymmetric unit by less than the maximum distance selected for the calculation. For crystalline materials, space group transformations and unit cell translations are used to generate the atom positions in the second sum.

The pair correlation function, $G(r)$, for a spherical region of radius t is defined as:

$$G(r) = 4\pi r [\rho(r)/\epsilon(r, R) - \rho_0] \epsilon(r, t)$$

where for spherical particles $\epsilon(r, R)$ is given by:

$$\epsilon(r, R) = 1.0 - \frac{3}{4} \left(\frac{r}{R} \right) + \frac{1}{16} \left(\frac{r}{R} \right)^3 \quad \text{for } r \leq 2R$$

$$\epsilon(r, R) = 0 \quad \text{for } r \geq 2R$$

When the input model is an infinite crystal, $\epsilon(r, R) = 1$ and $\epsilon(r, t)$ corrects for the fall-off in scattering near the diameter of a finite spherical particle. R is radius of model fragment.

The disorder parameters $\sigma_{ij} = 0.05$ at 1.5 Å and 0.19 at 10 Å were based on experimental values for SiO₂ glass obtained by Konnert and Karle³⁴ and Konnert *et al.*³⁵. The RDFs of the Al₃O₁₄ clusters were obtained using the published atomic coordinates of jadeite³⁶, chantalite³⁷ and Al₂(OH)₂TeO₃SO₄³⁸.

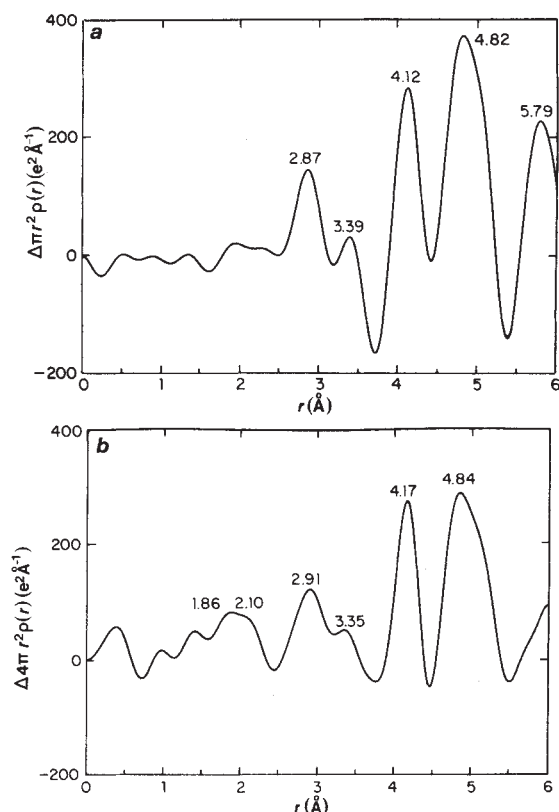


Fig. 2 RDFs for non-framework Al species in HY-DHV (a) and HY-DHSt (b).

Figure 1a shows a plot of $4\pi r^2 \rho(r)$ against r for H-Y prepared at 300 °C in vacuum. The peaks were identified by comparison with calculated distances from the structure refinement of $\text{H}_{53.8}\text{Na}_{2.2}\text{-Y}$ by Gallezot and Imelik³⁹. The T-O value of 1.653 $\text{\AA}$ agrees well with the mean T-O distance of 1.648 $\text{\AA}$ calculated for $\text{T} = \text{Al}_{0.29}\text{Si}_{0.71}$ from ionic radii⁴⁰.

The RDF of HY-DHV (Fig. 1b) shows significant changes in peak position and poorer resolution of the T-T and O-O peaks at 3.13 and 2.69 $\text{\AA}$ and 4.39 and 3.86 $\text{\AA}$, respectively.

More useful information is obtained, however, from subtraction of the HY RDF from the HY-DHV RDF. Figure 2a shows that this difference spectrum is characterized by peaks at 2.87, 3.39, 4.12, 4.82 and 5.79 $\text{\AA}$. Because NMR studies of Freude *et al.*^{19,20} show that extensive framework de-alumination occurs on dehydroxylation of H-Y at 650 °C in vacuum, we attribute the difference spectrum to the non-framework aluminium species.

To confirm that the difference spectrum of Fig. 2a is not an artefact, a further difference spectrum was obtained from the sample of Y dehydroxylated in steam at 890 °C (HY-DHSt) (Fig. 2b) using the same H-Y as a reference³⁰. The same prominent peaks as in Fig. 2a are present, but two additional peaks occur at 1.86 and 2.10 $\text{\AA}$. It is apparent that a similar residual species is present in both HY-DHV and HY-DHSt.

The Al-O peak at 1.9 $\text{\AA}$ is only barely visible in HY-DHV. Although visible in HY-DHSt, its intensity is less than predicted from the calculated RDF. The increased intensity of the 1.9- $\text{\AA}$ peak and the generally narrower peaks for HY-DHSt probably reflect the increased degree of condensation and crystallinity of the NFA species in ultra-stable Y.

Table 1 summarizes the RDF peak positions from the NFA

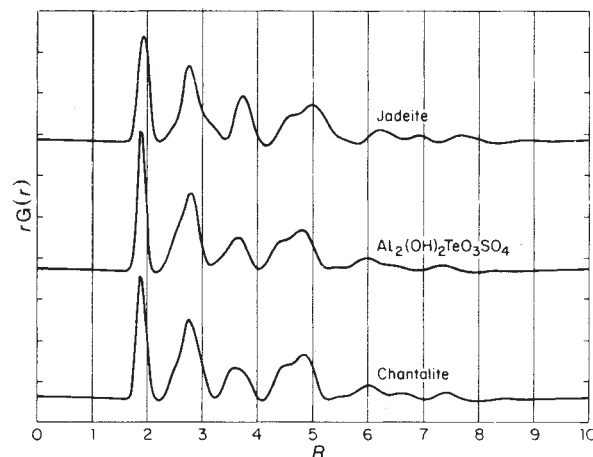


Fig. 3 Calculated RDFs for Al_3O_{14} clusters.

species in the two de-aluminated HY samples. The distances present in the NFA produced at 650 °C clearly correspond to a larger Al-OH cluster than the sample species listed earlier, that is, $\text{Al}(\text{OH})^{2+}$, $\text{Al}(\text{OH})_2^+$ or $[\text{Al}_2\text{O}]^{4+}$.

To help identify the NFA species, we have calculated the RDFs of $\text{Al}_3(\text{O},\text{OH})_{14}$ groups extracted from the edge-shared octahedral chains in jadeite, chantallite and $\text{Al}_2(\text{OH})_2\text{TeO}_3\text{SO}_4$. From the RDFs shown in Fig. 3, it can be seen that edge-shared octahedral clusters result in RDF peaks at positions similar to those shown in Table 1. The most prominent peaks occur at 1.9 $\text{\AA}$ (Al-O), 2.8 $\text{\AA}$ (O-O, Al-Al), 3.5 $\text{\AA}$ (Al-O), 3.8 $\text{\AA}$ (O-O), 4.4 $\text{\AA}$ (Al-O), 4.8 $\text{\AA}$ (O-O), 5.5 $\text{\AA}$ (O-O) and 5.9 $\text{\AA}$ (Al-O), where the values in parentheses indicate atom pair interactions calculated separately. This similarity suggests that the NFA species in HY-DHV and HY-DHSt contain edge-shared octahedral $\text{Al}_m\text{O}_n(\text{OH})_p$ chains.

Two significant differences exist between the NFA peaks and those of the $\text{Al}_3(\text{O},\text{OH})_{14}$ clusters. First, the NFA peak at 1.9 $\text{\AA}$ is considerably broader and less intense than the 1.9- $\text{\AA}$ peak in the chains. The breadth could result from the considerable disorder caused by a large number of terminal OH groups expected in 10- $\text{\AA}$ clusters located in the supercages of Y zeolite. The peak at 1.86 $\text{\AA}$ probably corresponds to terminal OH groups whereas the peak at 2.1 $\text{\AA}$ would correspond to bridging OH or O atoms. Second, the NFA peak at 4.1 $\text{\AA}$ is shifted considerably from the typical value of 4.5 $\text{\AA}$ for the $\text{Al}_3(\text{O},\text{OH})_{14}$ clusters and its intensity is greatly increased. This suggests a different arrangement of the chains from the simple isolated species used for the calculations. Further calculations of RDF spectra for other $\text{Al}_m\text{O}_n(\text{OH})_p(\text{OH}_2)_q$ clusters and the known crystalline forms of Al oxides and hydroxides are in progress with the hope that one of these will correspond to the difference RDFs of Fig. 2.

It should be emphasized that the NFA species formed in this study corresponds to an advanced stage of de-alumination under shallow-bed conditions. Dehydroxylation of H-Y occurs at 450 °C in shallow bed conditions and 300 °C in deep-bed conditions^{19,20}. The de-alumination carried out in this study is in the region of substantial dehydroxylation and therefore de-alumination. No attempt has yet been made to look at the de-alumination species obtained at lower temperatures. The species formed at 300-550 °C may well be different and could correspond to some of the species proposed to form before condensation as emphasized by Freude *et al.*¹⁹.

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Table 2 Peak positions in calculated partial RDFs for $\text{Al}_m\text{O}_n(\text{OH})_p$

$\text{Al}_m\text{O}_n(\text{OH})_p$	Compound	Al-O	O-O	Al-Al	Al-O	O-O	Al-O	O-O	O-O	Al-O	O-O
Al_3O_{14}	Jadeite	2.00	2.80	3.05	3.70	3.85	4.55	5.00			6.20
$\text{Al}_3\text{O}_{10}(\text{OH})_4$	$\text{Al}_2(\text{OH})_2\text{TeO}_3\text{SO}_4$	1.90	2.80	3.00	3.60	3.80	4.40	4.85		6.00	
$\text{Al}_3\text{O}_6(\text{OH})_8$	Chantallite	1.90	2.80	3.00	3.55	3.80	4.45	4.85	5.50	6.00	6.10

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Kilometre-scale sheath fold at Mattmark and implications for transport direction in the Alps

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Folds were the first tectonic features of rocks to be studied and as such are taken as a reference for the interpretation of other structures. Intuitively, fold axes were assumed to be subperpendicular to the direction of transport. Nevertheless, many studies of metamorphic belts showing intense ductile deformation describe folds with axes close to the transport direction. In particular, sheath folds¹⁻⁹, are highly curvilinear folds elongated parallel to the direction of transport. We discuss here a spectacular large sheath fold situated in a crustal shear zone in the Monte Rosa nappe, Swiss Alps. Our analysis implies that both the geometry and significance of folds in the internal Alps must be re-examined. We further demonstrate the existence of crustal ductile shear zones in an area of the Alps where previously emphasis was generally placed on fold nappes; the movements on these shear zones is often tens of kilometres.

The Monte Rosa nappe (Fig. 1), Swiss Alps, representing the northernmost internal crystalline massif, is a spectacular example of deep ductile deformation. It is made up of Hercynian basement rocks, mainly gneiss and schists, intruded by late

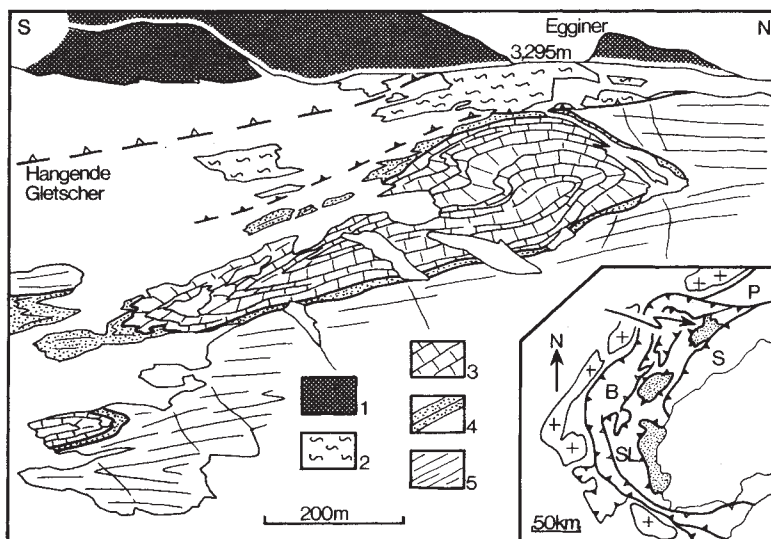
Hercynian granites¹⁰ and unconformably covered by Permo-Carboniferous volcanoclastic rocks and by a series of Mesozoic sediments¹⁰⁻¹². All these were intensively reworked during the Alpine tectonometamorphism with the formation of gneisses, micaschists and mylonites, and are tectonically overlain by the Zermatt Saas-Fee ophiolite thrust sheet¹³.

The polyphase Alpine metamorphism may be divided into two major events: as a high-pressure eclogite metamorphism^{14,15}, (the climax of this metamorphism occurred around 110 Myr ago in eclogitic conditions (15 kbar), followed by a decrease of pressure to 7-8 kbar until 60 Myr)^{16,17}, and the Alpine (s.s.) metamorphism at around 38 Myr ago^{17,18}, in greenschist to amphibolite conditions.

In the Monte Rosa area, as in all the pennine Alps, the intensity of the Alpine deformation is evidenced by large recumbent nappes^{19,20} and the presence of a prominent Alpine foliation and intense microfolding^{10,21-23}. However, such studies can only analyse fold geometry and usually describe several phases of folding²⁴. The direction of transport is generally assumed to be perpendicular to the fold axes and the vergence of 'tectonic phases' is deduced from the apparent vergence of folds.

Our recent work^{25,26} has attempted to study the deformation mechanisms using tectonic and microtectonic analysis. We obtained the following results: firstly, The most significant microstructure is the prominent stretching lineation. This is clearly recognized by the elongation of deformable markers (such as pebbles), the stretching of porphyroclasts or rigid inclusions (feldspars in gneissic rocks, dolomitic layer in marbles) and also by mineral elongation (biotite, phengite, recrystallized quartz, feldspar or calcite, elongation of pressure

Fig. 1 Geological landscape of the 'eye fold' of Mattmark, viewed from the Gruenbergorn (3,015 m) towards the north-west. 1, Ophiolites; 2, Jurassic brown calcschists; 3, marbles; 4, quartzites; 5, paragneiss and schists. Inset location of the studied area (Monte Rosa nappe) in the Western Alps. The internal crystalline massifs are shaded; crosses indicate the external crystalline massifs. B, Briançonnais zone; SL, Schistes lustrés and ophiolites; S, Sesia zone; P, pennine nappes of the Lepontine area.



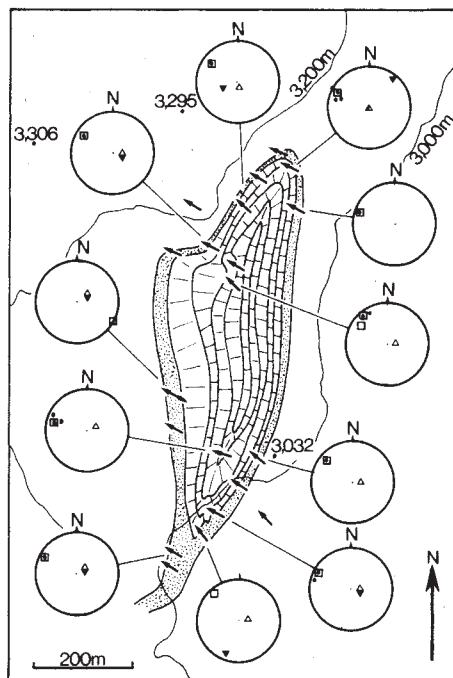


Fig. 2 Structural map of the 'eye fold'. The arrows represent the stretching lineations. In the Schmitt diagrams (lower hemisphere projection): Δ , foliation; $\square$, stratification; $\square$, stretching lineations; $\bullet$, intersection lineations (S_0/S_1 or S_0/S_{1-2}).

shadow crystallizations). These lineations represent finite extension. On average they trend N 110° E, plunging slightly to the west-north-west. Despite variations (from N 70° E to N 140° E) which may be related to heterogeneities of the deformation²⁷, they are regionally coherent. Secondly, the major deformation observed is generally intense and progressive, and shows evidence of non-coaxial deformation such as quartz petrofabrics²⁸, coherent displacement along shear zones²⁷, deformed porphyroclasts and pressure shadows²⁵, and shear band cleavages. Overall characteristics, the deformation resembles the result of simple shearing. As in shear zones the stretching lineation in the intensely deformed areas seems to lie close to the direction of shear.^{29,30} On a regional scale, the lineations trace approximately straight paths. Two different units can be distinguished on the basis of the determined shear senses²⁵. The first, the Monte Rosa nappe and its sedimentary cover, is characterized by shearing to the west-north-west associated with crustal ductile and major thrusting towards the west-north-west. The second unit, the Portjengrat unit, situated immediately south of the Mischabel backfold, shows east-south-eastward shears. We believe this deformation to be related to antithetic ductile thrusting³¹. Finally the observed progressive deformation mainly occurred during retrograde metamorphism at about 40 Myr. It may represent a late, although intense, phase of a complex deformation history and is probably related to major thrusting during and after continental collision.

In the vicinity of Mattmark in the Saas valley, a large-scale 'eye fold' has been recognized³² and studied²⁵. It is well exposed (Fig. 1), between 2,700 and 2,200 m, in a cliff oriented NNE-SSW and has been partly mapped^{11,21}. It affects the sedimentary rocks of the Gornegrat zone with Upper Triassic marbles in the core of the fold, surrounded by Lower Triassic quartzites. This structure is in turn surrounded by schists and paragneisses (probably Permo-Carboniferous) and overlain by a slice of brown calcschists (*schistes lustrés* of supposed Jurassic age) and by the Zermatt Saas-Fee ophiolitic thrust sheet¹³.

From the neighbouring summits (around 3,000 m) the 'eyed' structure is picked out by marble layers. On the outcrop both quartzite and marble layers surround the eye without discontinuity and define a closed structure (Fig. 1). The stretching lineation is generally prominent; in quartzites, very elongated

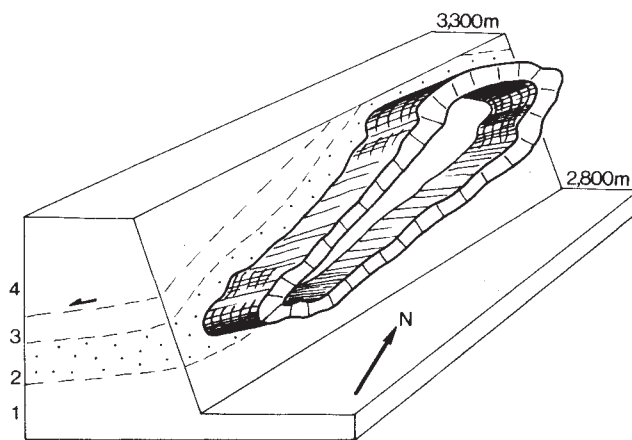


Fig. 3 Schematic structure of the 'eye fold'. 1, Paragneiss and schists; 2, quartzites; 3, calcschists; 4, ophiolitic thrust sheet (mainly serpentinites).

pebbles, quartz rods and a mineral lineation (elongated phengite or quartz crystals) can be seen; in marbles or calcschists, the lineation is marked by boudinage of the dolomitic layers, the stretching of dolomitic inclusions or porphyroclasts (such as garnets) and elongation of minerals, and pressure shadows. These lineations show nearly constant direction in the studied area (Fig. 2) and on average trend N 120° E. Microfolds are isoclinal and more frequent in the north and south hinge zones of the eye fold (where we also observed small sheath folds and a mushroom-shaped fold) and also on the Jurassic calcschists. Both the fold axes (microfolds, north and south hinge zones of the eyed structure) and the intersection lineation, S_0/S_1 or S_0/S_{1-2} , are parallel to the stretching lineation (Fig. 2).

The relative orientations of the bedding (S_0) and the fold axes suggest that the fold is cylindrical and that the eyed pattern is not a topographic effect (Fig. 3). Based on this structure and on the intensity, mechanism and kinematic of deformation, we interpret this eye fold to be a section lying subperpendicular to the axis of a large sheath fold^{1,2}. This fold would be elongated parallel to the stretching lineation trend and of the order of several kilometres in size. It represents a synclinal structure with the younger rocks (marbles) in the core. There is no direct evidence for an east or west closure of this fold, that is, an eastward- or westward-facing nose. It is possible that a such large sheath fold is the result of a dramatic deformation associated with the westward crustal shearing rather than ductile backthrusting; if this is the case, the closure of the synclinal sheath fold should be to the east.

Other large folds exist in the Gornegrat zone. For instance, immediately north of the eye fold, we have described a refolded isoclinal fold of kilometre scale whose axis is always parallel to the stretching lineation²⁵. On the metric scale, most folds show certain characteristics consistently over the whole area studied: they are highly isoclinal with very elongated limbs; the fold axes are generally straight and parallel to the stretching lineation; folds are clearly visible in sections perpendicular to the lineation where their apparent vergence is generally to the south-west. Nevertheless, folds showing opposite vergence (both to the south and north) on the same outcrop and mushroom-shaped folds are relatively common. Two major folding phases (F_1 and F_2) with the same geometrical characteristics have previously been described²².

Hence, the present deformation analysis indicates that folds in the Monte Rosa area (except obvious late folds) may represent either: (1) folds ('a folds')³³ with axes close to the long axis of the strain ellipsoid, as shown by the stretching lineation, which then approach the shear direction or (2) sheath folds; both result from or were strongly deformed during, the large progressive shearing.

The mechanism of formation of sheath folds during progressive deformation is well established^{1,2}. They appear to be the

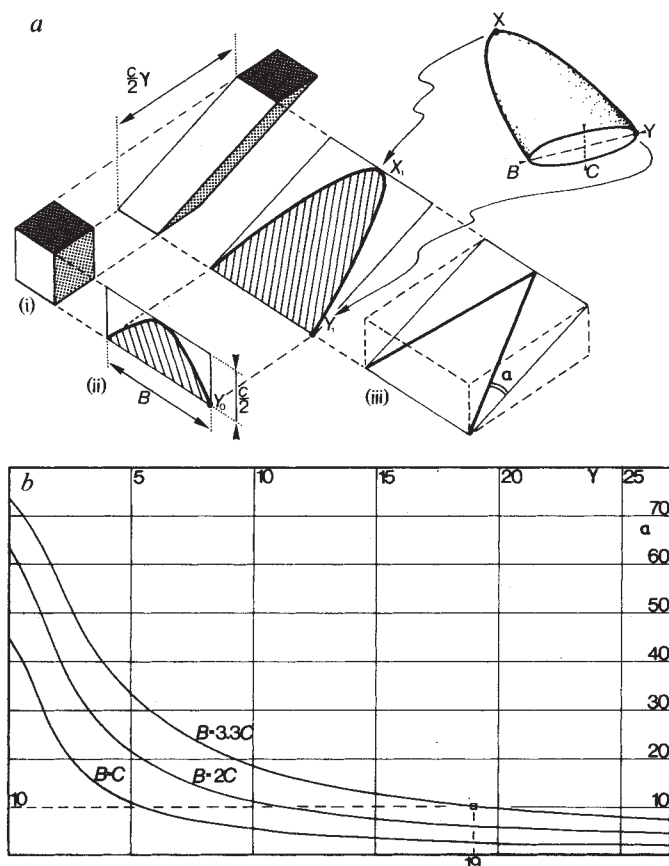


Fig. 4 Progressive reorientation of the limbs of a passive sheath fold. *a*, The model. (i) Idealized deformation of a cube of side $C/2$ by simple shear (with shear strain γ); (ii) the same deformation applied to a cross-section of shape B, C representing the hinge zone of the fold; (iii) a simplification of this geometry to yield the angle α between the limbs and the long axis of the fold, as a function of γ and of the shape (B/C) of the fold. *b*, Curves of α versus γ . The curve $B = 3.3C$ corresponds to the fold of Mattmark. γ is estimated to a minimum value of 19.

result of kinematic amplification of 'deflections' in the folded layers which produced curvilinear folds that became progressively more elongated during the deformation. The presence of a kilometre-scale sheath fold suggests that the shearing mechanism took place over a large area and caused very large deformation and transport. Commonly occurring folds lying parallel to the direction of shear were probably formed by reorientation, during the progressive deformation, of folds initially lying oblique to the shear direction^{30,34}. Such oblique folds may have been induced during this deformation by the shearing of layers inclined relative to the shear plane^{35,36}.

In the absence of strain markers, an estimate of the shear strain can be obtained from a simple model of the geometry and evolution of the sheath fold. If exact, such an estimate would be more significant than a point-by-point strain analysis, because it would represent the average bulk shear strain for the whole volume of rock.

Assuming simple shear for the progressive deformation (Fig. 4*a*(i)), and a passive sheath fold, the shape of the hinge zone (Fig. 4*a*(ii)), represented in Fig. 4*a*(iii) by a triangle, is considered to be the result of kinematic amplification² of the deflection (Fig. 4*a*(ii)). We may calculate the angle α as a function of the shape of the sheath fold (B/C) and the shear strain (γ):

$$\alpha = \arctan \left(\frac{B}{C} \frac{1}{\sqrt{\gamma^2 + 1}} \right) \quad (1)$$

Different curves, for different values of B/C , can now be plotted (Fig. 4*b*); the curve $B/C = 10/3$ corresponds to the Mattmark fold. We may then relate the characteristics of the observed

sheath fold (the angle between the fold axes in the two hinge zones of the eye, which corresponds to 2α). Based on the approximately cylindrical shape of the Mattmark fold, we can consider the angle between the two axes to be $<20^\circ$ (that is, $\alpha \leq 10^\circ$). This leads to a minimum estimate for the bulk shear strain of $\gamma = 19$, for a fold with the observed dimension ($B/C = 10/3$). Although this is an oversimplified model (which assumes simple shear, unknown shape of the deflection, and only passive reorientation), such an estimate may be a useful average value for the bulk shear strain. It agrees with the microstructural and textural characteristics of rocks such as: the elongation of quartz pebbles in conglomerates²⁵ (λ_1/λ_3 up to 50 and a K parameter close to 1 where λ_1 and λ_3 are finite strain axes and K is the shape parameter for the strain ellipsoid and is 1 when in plane strain); mylonitic microstructures³⁷; and well-defined preferred lattice orientation of quartz.

In terms of transport, for a shear zone 1 km thick (minimum thickness for the intensely sheared area situated at the top of the Monte Rosa nappe), the value for γ obtained requires a minimum displacement of ~ 20 km. Note that much more movement may occur on discrete fault zones.

Such an analysis, associating folds with a deformation mechanism, has several implications.

First, for highly deformed areas, it seems logical to assume that folds perpendicular to transport ('b fold') cannot exist except in the case of late folds. This is true for mylonite zones³⁸ and also for deep parts of mountain belts such as the Himalayas³³, the Alps^{39,40} or the North American Cordillera^{41,42}. Hence, geometrical analyses of folds are not good kinematic indicators and must be used in association with analyses of ductile deformation mechanisms.

Second, this work demonstrates the importance of thrust displacements of several tens of kilometres along ductile shear zones, in an area where previously much emphasis was placed on fold nappes^{19,22-24}. As has already been proposed for the French-Italian Alps⁴⁰, deformation analyses show that the evolution of the Swiss part of the belt is highly logical, and is dominated by thrusting towards the west-north-west, without major gaps between the external and internal Alps.

Finally, deformation analysis should be carried out on all the internal parts of the Alps. Some advances have been made on the French-Italian part^{43,44}, but in the famous Pennine nappes of the Swiss Alps^{19,20} studies have concentrated on the superposed folding^{23,24,45}. According to the highly deformed state of the rocks, fold geometry seems to be unrelated to the kinematic evolution, except in the case of late folds. It is probable that major deformations occurred in a shear context^{46,47} with the formation of stretching lineations^{48,49} tracing the shear directions. It would now be of great interest to study ductile deformation and its kinematics and to relate it to the fold geometries, with a view to analysing sheath folds⁴⁷ and folds subparallel to the shear directions.

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An early Cambrian rift to post-rift transition in the Cordillera of western North America

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The upper Proterozoic and lower Palaeozoic wedge of miogeoclinal strata in the North American Cordillera is widely regarded as evidence for a proto-Pacific passive margin^{1–3}. The rifting history of this margin appears to have been protracted, possibly spanning >200 Myr in a tectonic setting that is not well understood³. Quantitative subsidence analyses of lower Palaeozoic strata between British Columbia and Utah, however, provide indirect evidence that the transition from rifting to post-rift cooling occurred within a relatively short interval of time, although probably not synchronously, between 600 and 555 Myr (refs 4–6). This age is significantly younger than that implied in previous studies^{1,2}. We describe here new field data, which, together with published geological data, provide direct evidence of a latest Proterozoic or early Cambrian age for the rift to post-rift transition. The data support recent arguments for widespread initiation of passive margins around the edge of the North American craton close to the Cambrian–Precambrian boundary⁷.

Indirect evidence for the ages of the rift to post-rift transition as well as for the final rifting phase were inferred from tectonic subsidence curves for early Palaeozoic strata in the Cordilleran miogeocline^{4–6} (Figs 1, 2). The strata (ranging from early Cambrian to late Ordovician in age) are laterally continuous, stable-shelf deposits and are regarded as part of an extensive post-rift continental terrace^{1–3}. The tectonic subsidence curves have the same form as the age–depth curve for ocean floor (Fig. 2). This indicates that, as in modern passive margins, the post-rift tectonic subsidence was controlled mainly by contraction of the lithosphere resulting from the decay of thermal anomalies generated during rifting^{4–6}. We emphasize, however, that the curves do not distinguish between margins adjacent to an open ocean or a back arc basin. The steep slopes of the curves, which are a function of the rates of heat loss, place the maximum age for the beginning of post-rift cooling at ~600 Myr (refs 4–6). This is because older ages for initiation of post-rift cooling, at 650 Myr, for example, would require anomalously high rates of heat loss from the Cambrian ocean floor, reaching at least 160%

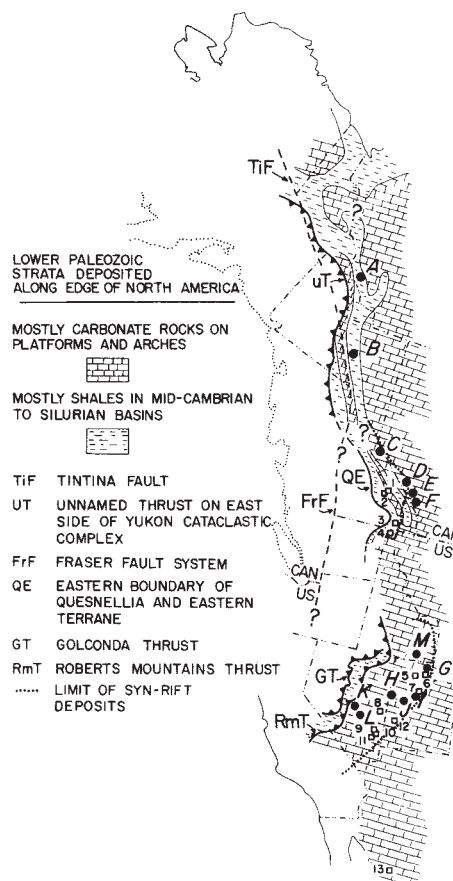
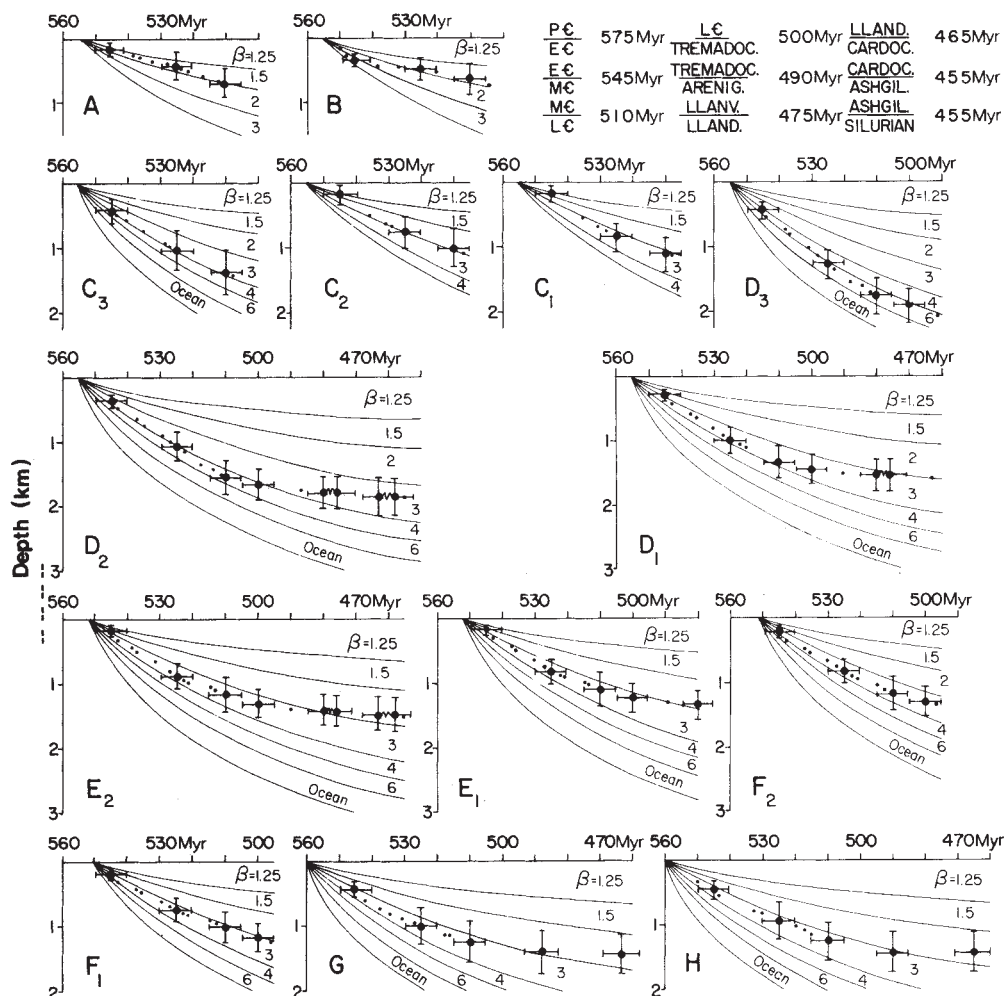


Fig. 1 Distribution of the lower Palaeozoic miogeoclinal facies in western North America (modified from ref. 5). UT, QE, GT and RMT are major structural boundaries of various terranes accreted to western North America in post-early Palaeozoic time³. These boundaries mark the westernmost preserved edge of the lower Palaeozoic North American continent. Ti–FrF is a major right-lateral transcurrent fault system³. The dotted outline of western North America gives the position of this region after restoration of the right-lateral transcurrent movement on the Tintina–Fraser fault system⁵. Heavy dotted lines indicate the approximate eastern limits of the latest Proterozoic or earliest Cambrian post-Windermere rift deposits in the southern Canadian Rockies and in Utah–Nevada. ●, Locations of sections in which tectonic subsidence has been calculated^{4,5}. □, Locations where mafic extrusive rocks occur in post-Windermere rift deposits that record the final (latest Proterozoic or early Cambrian) rifting in the margin. Sources: 1, 2, 3, Hamill Group^{15,16,35}; 4, Three Sisters Formation (although the volcanics may be in the Windermere Supergroup, F. Miller, personal communication); 5, Mutual Formation (Crittenden, unpublished mapping); 6, Browns Hole Formation²³; 7, Tintic Quartzite³⁰; 8, 12, Prospect Mt Quartzite^{26,29}; 9–11 Stirling Quartzite²⁶; 13, Sonora^{33,34}.

of that of the modern ocean floor⁵. The high rates of post-rift cooling further indicate that relatively little heat was lost during rifting, and in terms of finite rifting models⁹, the duration of the final rifting phase probably did not exceed 10–20 Myr. The age of final rifting, therefore, could not be much older than our inferred maximum estimate for the rift to post-rift transition.

The subsidence data do not preclude earlier extensional and heating events, which are indicated by geological evidence and have profoundly influenced the structural evolution of the margin, probably since the Middle Proterozoic^{2,3,10}. However, igneous rocks thought to be associated with late Proterozoic rifting in the margin have radiometric ages of ~900–750 Myr (refs 8, 10, 11), and clearly are too old for the final rifting of the margin. Our mapping in the southern Canadian Rockies and in Utah, together with a synthesis of published data, have led to the identification of a widespread younger rifting event

Fig. 2 Tectonic subsidence (●) for sections located in Fig. 1 compared with thermal subsidence curves from the McKenzie stretching model³⁶ (solid curves). Large dots are stratigraphical boundaries to which numerical ages have been assigned. Small dots are formation boundaries positioned by assuming constant sedimentation rates between dated boundaries. Vertical bars give the range between maximum and minimum values of tectonic subsidence⁴. Horizontal bars give an assumed ± 5 -Myr error for stratigraphical correlations with numerical ages. β , Crustal stretching factors from the McKenzie stretching model; β values corresponding to the subsidence curves are not necessarily the stretching factors for the passive margin. Letters with subscripts designate different sections lying along east-west lines at locations that are too close to indicate separately in Fig. 1. The higher subscript numbers designate the more westerly sections. Most of the sections with subscripts are >15 km apart on a non-restored base. Numerical ages assigned to the stratigraphical boundaries²⁰ are shown in the upper right-hand corner. Stratigraphical boundaries are from ref. 37.



whose age appears to be compatible with the high rates of post-rift cooling in the margin.

In the southern Canadian Rockies, limited evidence for younger rifting has been reported from the Hamill Group west of Golden^{4,12} and from the McNaughton Formation near Jasper¹³ (Fig. 3). Both deposits underlie strata with early Cambrian trilobites and overlie the Windermere Supergroup (Fig. 4a), a widespread stratigraphical unit that was deposited during rifting in late Proterozoic time (refs 2, 3, 10, 11). Both the Hamill Group and McNaughton Formation contain distinctive thick sequences of feldspathic sandstones and conglomerates¹³⁻¹⁶ (Fig. 4a). In the Hamill Group west of Golden (Fig. 3), shallow-marine sediments pass abruptly into deeper marine deposits that contain resedimented sandstones, pillowed lavas and mafic volcanoclastic debris^{12,15,16}. These facies changes are regarded as evidence of syn-depositional faulting accompanied by extrusion of mafic rocks¹². Similarly, near Jasper, abrupt changes in thickness in the McNaughton Formation have been interpreted as evidence of syn-depositional faulting¹³. Strata of the Hamill Group and the McNaughton Formation pass gradually upwards into the lower Cambrian mature quartz sandstones with *Skolithos* that are part of the early post-rift strata of the margin (Fig. 4a). Neither, however, is overlain by post-rift strata that are suitable for quantitative analysis, and their precise stratigraphical position relative to a rift to post-rift transition cannot be inferred from subsidence data.

Recent field work in areas east and south of the exposures of the McNaughton Formation and Hamill Group (between $51^{\circ}45'$ and $52^{\circ}30'$, Fig. 1) has indicated that at least two of the sections analysed, SF and MtWi, and probably HL7b and GL as well (Fig. 3), are underlain conformably by a rift-like deposit that is at least 500 m thick. This deposit forms a distinctive basal unit

of the Gog Group and is broadly correlative with the McNaughton Formation and at least the lower part of the Hamill Group¹⁷ (Fig. 4a). The exact stratigraphical relation of this deposit to formally recognized strata in the region is not yet established, however, and we refer to it informally as a basal conglomeratic unit of the Gog Group.

A rift origin of the basal conglomeratic unit is suggested by a striking succession of cross-bedded, poorly sorted arkosic to subarkosic sandstones and conglomerates interbedded with thin layers of red shales and siltstones in the lower two-thirds of the unit (Fig. 4a). The sandstones and conglomerates contain ~30–80% quartz, 15–50% feldspar, more than half of which is a distinctive pink alkali feldspar as much as 2 cm across, and 5–20% rock fragments consisting mainly of quartzite, chert and granitic clasts. A total of 219 measured orientations of trough axes and large-amplitude (50 cm to 2 m) cross-beds, corrected for tilting, indicate mainly unimodal westerly current flow. The coarse grain size, arkosic composition, abundance of red shales together with westerly palaeocurrent directions suggest a fluvial or alluvial fan environment and a strongly uplifted source in crystalline basement to the east. An easterly provenance in crystalline basement has also been suggested for the McNaughton Formation based on palaeocurrent measurements and sandstone compositions^{13,18}. The abrupt termination of the conglomeratic unit between Mt Chephren and Bow Pass (Figs 3, 4a) could reflect deposition against a fault scarp or hinge zone flanking the easterly basement source. The conglomeratic unit of the Gog Group grades upward into mature quartz sandstones with *Skolithos*, which are correlative with the post-rift deposits in the upper part of the McNaughton Formation and in or above the upper part of the Hamill Group (Fig. 4a). These *Skolithos*-bearing strata form the base of the sections used in subsidence analyses.

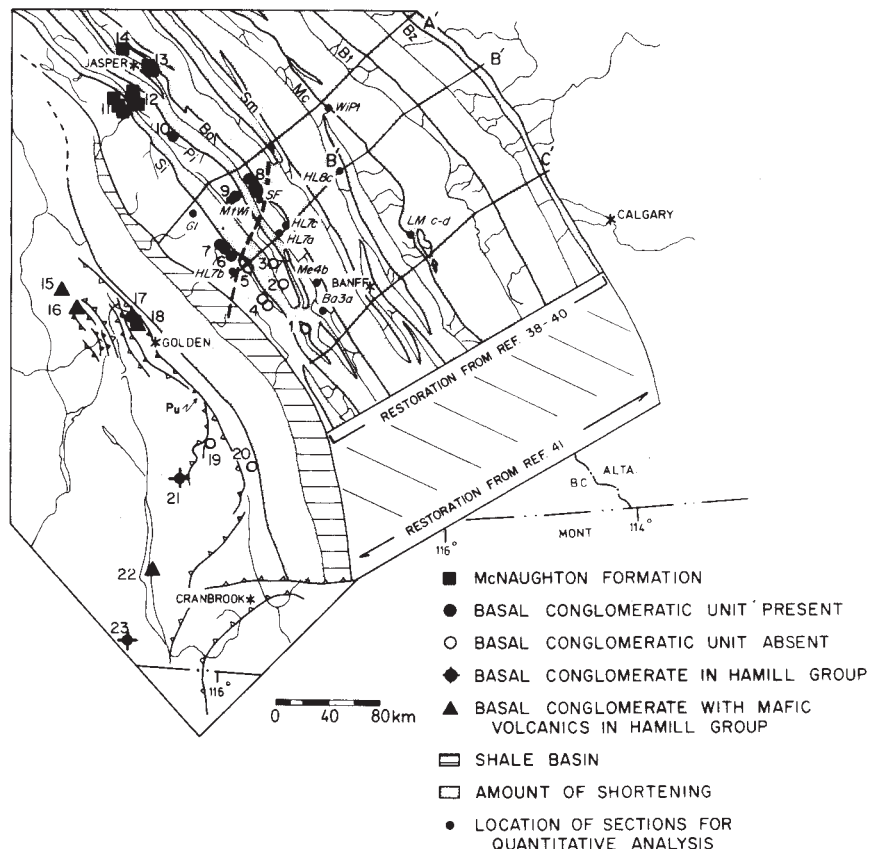


Fig. 3 Distribution, on a palinspastic restoration, of the uppermost Proterozoic to lowest Cambrian strata that are interpreted to record the final rifting and the transition to post-rift subsidence in the southern Canadian Rockies. Large filled circles, solid circles, solid circles with cross, solid squares, open circles and solid triangles mark localities examined in the field. Open circles west of Banff are the Gog Group and south of Golden are the Cranbrook Formation. Stippled area is the amount of shortening between thrust panels that are unpatterned. The shortening in the shale basin has not been determined from balanced sections and is assumed here to be equal to the amount of shortening in the carbonate bank (a factor of 2). The displacement on thrusts west of the shale basin has not been estimated. Italic letters correspond to analysed sections shown in Fig. 1. *WiPi*, *SF*, *MtWi*, *GL* are at locality D, Fig. 1; *HL8c*, *HL7c*, *HL7a*, *HL7b* are at locality E, Fig. 1, and *LMC-D*, *Me4b* and *Ba3a* are at locality F, Fig. 1. Lines A', B' and C' are lines of palinspastic restoration which are control for restored positions of thrust plates³⁸⁻⁴². The dashed line is the approximate position of the southeastern edge of the basal conglomeratic unit.

In the McNaughton Formation, the change from coarse feldspathic sediments to mature, marine quartz sandstones, which we assume is approximately correlative with the end of final rifting and the transition to post-rift subsidence, occurs in the lower part or below the early Cambrian *Fallotaspis* trilobite zone¹⁹ (Fig. 4a). The transition to post-rift subsidence, therefore, lies near the base of the Atabian Stage or in the Tommotian Stage. These stages are not well dated but are thought to range from slightly younger than 570 Myr to ~590 Myr (ref. 20). If the correlation of the McNaughton Formation with the basal conglomeratic unit and the Hamill Group suggested in Fig. 4a is even approximately correct, there is excellent agreement between the stratigraphical position of the rift to post-rift transition and the age of that transition inferred from the subsidence data.

There is also evidence, from work in progress, for syn-depositional rifting in the western United States, in uppermost Proterozoic and lower Cambrian rocks that are broadly correlative with the succession in the southern Canadian Rocky Mountains (Figs 1, 4b). The principal evidence for rifting is the occurrence of feldspathic sandstones and conglomerates, relatively abrupt lateral facies and thickness changes, regionally variable palaeocurrent directions and the presence of mafic volcanic flows and volcanoclastic conglomerates. Few chemical data are available for the volcanic rocks, but the rocks appear to be of tholeiitic to alkalic affinities, consistent with continental rifting^{1,21}. The feldspathic sandstones and conglomerates occur sporadically, but in places constitute units several hundred metres thick^{22,23}. Sequential changes in mineralogical maturity and grain size are locally abrupt, consistent with episodic uplift and exposure of crystalline basement. In Utah, for example, medium- to fine-grained, well-sorted, shallow-marine (?) quartzite of the upper Browns Hole Formation is overlain with sharp contact by arkosic, poorly sorted fluvial (?) conglomerate of the Geerts Canyon Quartzite²³ (Fig. 4b). In general, the occurrence of lateral facies and thickness changes tends to be obscured by formational nomenclature which emphasizes the regional persistence of gross stratigraphical units. However, in one well-documented example, the middle member of the Wood Canyon

Formation in southeastern California²², strata thicken and coarsen to the north-east and contain south-west-directed palaeocurrents, whereas associated stratigraphical units thicken to the west or north-west, and yield west- to north-directed and polymodal palaeocurrents^{24,25}.

The inferred rift deposits in Utah and Nevada overlie strata that are thought to be correlative with the Windermere Supergroup in the Canadian Cordillera^{26,27}, and they pass upward into lower Cambrian mature quartz sandstones with *Skolithos* that lie at the base of the post-rift strata from which the subsidence data were collected (Figs 1, 2, 4b). In the Delamar area of eastern Nevada, lithostratigraphical correlation with a section in the White-Inyo Mountains, California, suggests that the transition upward into post-rift mature quartz sandstones occurs near the base of or below the *Fallotaspis* trilobite zone^{26,28}. In the Wah Wah²⁹ and East Tintic³⁰ sections, the transition to post-rift strata is not well dated but seems to occur at about the same stratigraphical level relative to the base of the middle Cambrian as in the Delamar area²⁶. In the Huntsville section^{23,27}, the transition may be slightly lower stratigraphically but is probably broadly correlative with that to the south. In the western United States, therefore, as in the southern Canadian Rockies, the stratigraphical position of the final rifting to post-rift transition is in good agreement with the age of that transition inferred from the subsidence data.

The age of the final rifting episode can also be estimated from stratigraphical relations at several localities in the miogeocline. Near Jasper, in the southern Canadian Rockies, the uppermost 500 m of the underlying Windermere Supergroup (Miette Group, Fig. 4a) contains the trace fossils *Platysolenites* and *Didmaulichnus* and a microbiota with *Campitius* and *Volborthella* spp.^{13,31}. These are reported to occur in the Tommotian Stage (~570–590 Myr) and possibly younger stages of the early Cambrian^{13,32}, but their range is not well established. The rift deposit clearly lies above the Windermere Supergroup and only a short distance beneath probable earliest Cambrian strata, suggesting a latest Proterozoic age for the deposit. Similarly, in Utah and Nevada, the mafic volcanic rocks and feldspathic deposits occur a short distance below the *Fallotaspis* zone in

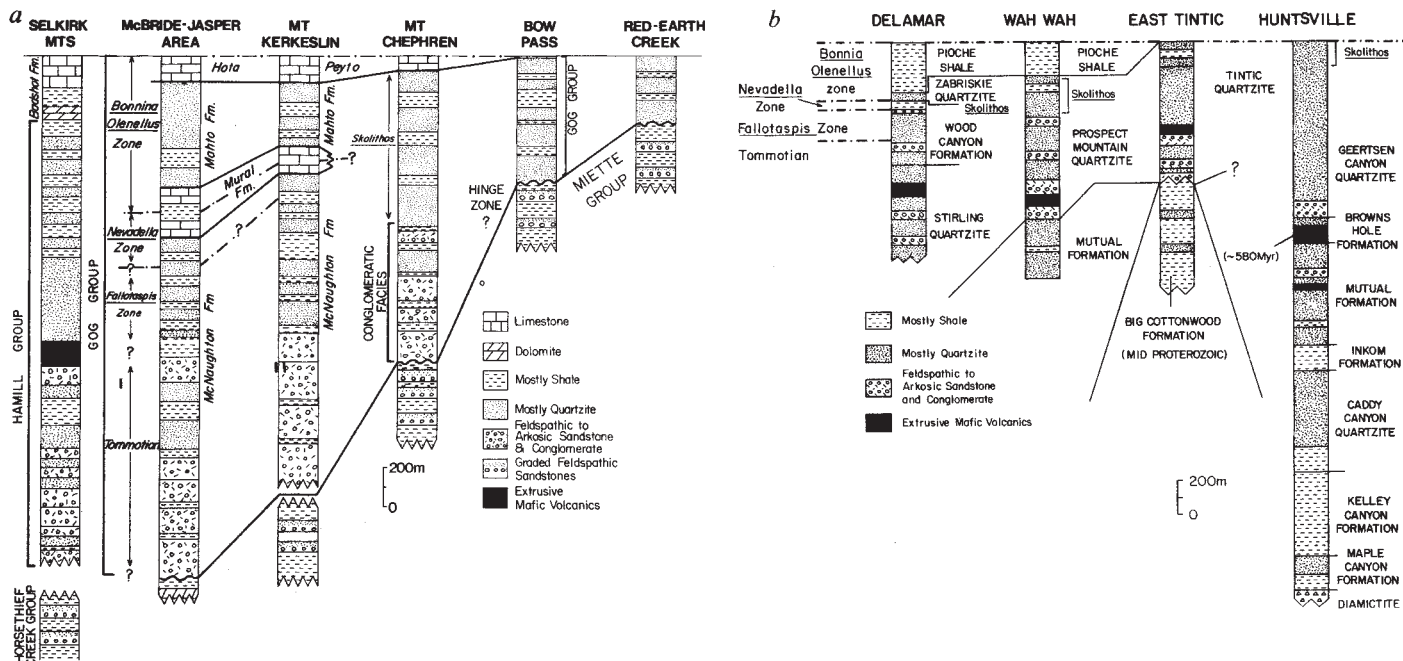


Fig. 4 *a*, Representative sections of the basal conglomeratic unit of the Gog Group and its probable equivalents in the McNaughton Formation and the Hamill Group. Columns show the transition of these strata to post-rift deposits of the miogeocline. These strata are interpreted as evidence of post-Windermere rifting and latest Proterozoic to earliest Cambrian transition to the post-rift phase in the margin. The Horsethief Creek Group and the Miette Group compose the Windermere Supergroup west and east of the Rocky Mountain Trench, respectively. Dash-dot lines are approximate boundaries between trilobite zones. The base of the middle Cambrian (uppermost dash-dot line) is the reference datum of the sections. The sections are located in Fig. 3 as follows: 1, Red Earth Creek; 5, Bow Pass; 7, Mt Chephren; 12, Mt Kerkeslin; 14, McBride-Jasper area; 17, 18, Selkirk Mts. Sources: Red Earth Creek, Bow Pass and Mt Chephren from G.C.B., Ma and M.A.K. (field work 1982 and 1983); Mt Kerkeslin from refs 13, 43; Selkirk Mts from ref. 16. Faunal zones are from ref. 19. *b*, Representative sections from Utah and Nevada indicating inferred final rift deposits and the transition to post-rift strata for comparison with similar successions in the southern Canadian Rockies. Dash-dot lines are approximate boundaries between trilobite zones. The ages in the Delamar section are based on lithostratigraphical correlation with biostratigraphically dated strata in the White-Inyo Mountains of California, about 300 km to the west. The upper part of the Stirling Quartzite is correlated with strata assigned a Tommotian age in California; the base of the Tommotian stage has not been established in the western United States³⁴. Sections located in Fig. 1 and sources as follows: 11, Delamar²⁶; 12, Wah Wah²⁹; 7, East Tintic³⁰; 6, Huntsville^{23,27}.

strata that are probably Tommotian in age (Fig. 4b), which, together with the 580-Myr Ar/Ar age from the volcanic rocks in northern Utah²¹, suggests that rifting occurred in the latest Proterozoic and/or earliest Cambrian. Finally, in Mexico, post-rift carbonate rocks and quartz sandstones of early Cambrian age pass gradationally downward into nearly 300 m of strata containing mafic lava and mafic volcanoclastic rocks. These volcanic deposits seem to be correlative with, or slightly older than, the lower part of the *Fallotaspis* zone^{33,34}.

Large parts of the Cordilleran miogeocline from Canada to Mexico, therefore, contain evidence of late Proterozoic rifting events that led to the formation of an extensive proto-Pacific passive margin. These events began perhaps as early as 800–900 Myr but appear to have terminated in a final, distinct and short-lived phase near the Cambrian–Precambrian boundary. The age is compatible with ages for the final rifting and rift to post-rift transition inferred from quantitative subsidence data. The results of the subsidence analyses were fundamental to this research, not only focusing on the problem of the ages for rifting, but also in predicting the stratigraphical position where the final rift deposit and the transition to post-rift strata should occur. This greatly facilitated the search for these deposits in the field. We have now identified these deposits in a large region in the southern Canadian Rockies and in an even larger area between southeastern Idaho and southeastern California (indicated by the dotted lines in Fig. 1). It seems that final rifting along a large portion of the proto-Pacific margin of western North America occurred at about the same time as rifting in the early Palaeozoic miogeocline in Arctic Canada and along an extensive portion of the lapetus margin in the US Appalachians and Newfoundland⁷. The contemporaneity of these events support recent arguments for rifting around nearly all of the perimeter

of North America close to the Cambrian–Precambrian boundary⁷.

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Direct measurements of secondary currents in a meandering sand-bed river

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Natural channels often adopt a meandering course. Water flow in meander bends is three-dimensional, consisting of primary velocities which are tangential to the bend, and secondary velocities, which are in the radial plane. The pattern of secondary flow strongly affects the distribution of erosion and deposition in the bend and the way in which the channel shifts and changes shape. Measurements of primary and secondary flows in a meandering gravel-bed river^{1,2} show that, in addition to the widely recognized main secondary circulation driving surface water outwards and bed water inwards, there can be a small cell of reverse rotation at the outer bank. Further data have been collected in a sand-bedded river at low, intermediate and high discharges. The results confirm the existence of the main and outer bank cells but also indicate that in some bends the main cell does not extend to the inner bank. In fact, secondary flow at the inner bank of wide, shallow bends is directed radially outwards over the whole flow depth at all in-channel flows. This indicates that some models of bend flow and channel development may be significantly in error.

Secondary currents are defined as currents that occur in a plane normal to the axis of primary flow³. In meander bends, they develop by skewing of cross-stream vorticity into a long-stream direction^{3,4}. The resulting skew-induced secondary circulation carries fast surface water towards the outer bank and slower bed water towards the inner bank.

At the outer bank, the primary flow, secondary circulation, and bank interact to produce a small cell of reverse rotation to the skew-induced cell. This cell occupies the channel to a distance one or two times the bank height away from the bank. Although this is a small proportion of the cross-section in most rivers, the outer bank cell is still important because it strongly affects the distribution of boundary shear stress, thereby influencing bank erosion processes^{5,6}.

In the central part of the channel, helical skew-induced flow produces inward velocities near the bed, which sweep bedload towards the inner bank. Sediment accumulation as a point bar at the inner bank gives the channel an asymmetrical cross-section. The balance between the transverse, upslope component of fluid drag on a bedload particle, and the transverse, downslope component of particle weight has been used as the basis for models of bed topography in bends^{7,8}. This view of secondary flow-point bar interaction has been challenged recently⁹. Dietrich and Smith suggest that secondary flow above the point bar is directed radially outwards over the whole flow depth and that the helical skew-induced cell is confined to the deepest, or thalweg, portion of the cross-section. Sediment accumulation is

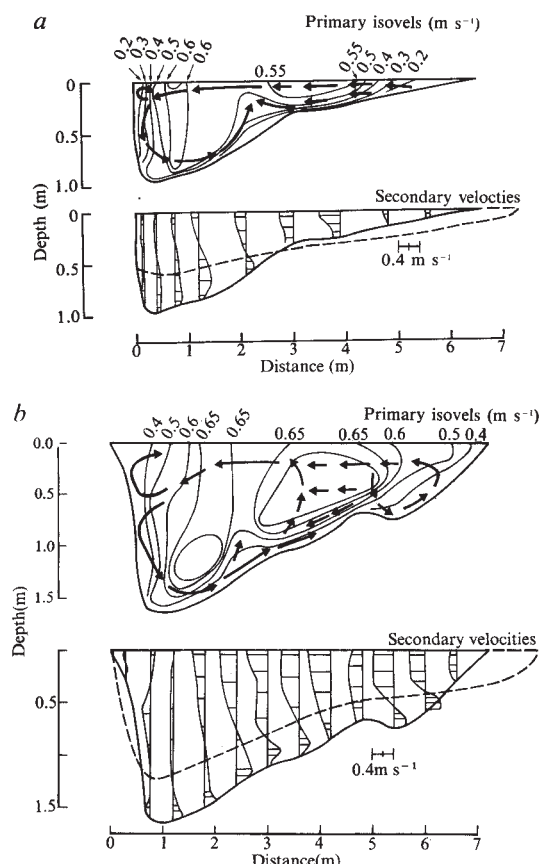


Fig. 1 Upstream bend apex, discharge: a, $1.7 \text{ m}^3 \text{ s}^{-1}$; b, $4.0 \text{ m}^3 \text{ s}^{-1}$. Dashed channel section represents that at the previous measurement section, $\sim 6 \text{ m}$ upstream.

then concentrated on a steep transverse slope called the point bar face, between the upper point bar (point bar platform) and the thalweg, where near bed flow converges and there is strong upwelling. Bridge¹⁰ suggests that this pattern of flow and point bar building is a characteristic of point bar emergence at flows below about two-thirds bankfull. He suggests that at such low stages the point bar topography causes flow convergence at the bend entrance, but that such patterns are replaced by helical flow at formative (that is, bankfull) discharge.

To help to resolve this discussion, data were collected in this study over a range of discharges in meander bends of the Fall River in Rocky Mountain National Park, Colorado. The reach studied has a bankfull capacity of about $4 \text{ m}^3 \text{ s}^{-1}$ and well developed meanders with a sinuosity of 2.2. The annual hydrograph is dominated by snowmelt runoff, resulting in long periods of almost steady bankfull flow in May, June and July. The gradient is $\sim 0.1\%$. The bed material is sand, $D_{50} = 1.0 \text{ mm}$, moving in dunes, ripples and as suspended load. Sediment availability has been greatly increased by erosion associated with the failure of Lawn Lake Dam on the Roaring River (an upstream tributary) on 15 July 1982. As a result, the bed is mobile at all discharges and point bars in the bends respond quickly to changes in flow stage.

Measurements were made at low, intermediate and bankfull stages, corresponding to discharges of 1.7 , 2.8 , and $4.0 \text{ m}^3 \text{ s}^{-1}$. Long and cross-stream velocities were measured using an electromagnetic current meter capable of measuring two mutually perpendicular velocity components with an accuracy of $\pm 3 \text{ mm s}^{-1}$. All measurements were made from temporary bridges aligned at right angles to the outer bank, and care was taken to work only over dune crests, avoiding separation zones on the lee side of dunes. Data were collected at 18 sections evenly spaced along the channel through two consecutive bends. The complete data set is available^{11,12} but because of limitations of space, only data for the intermediate and high flow at the

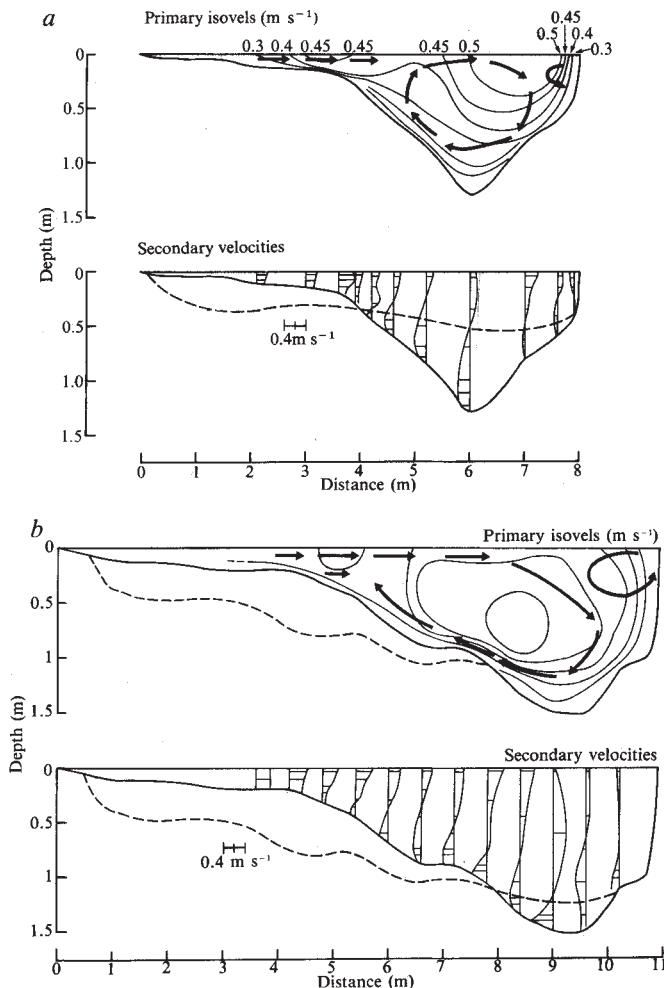


Fig. 2 Downstream bend apex, discharge: a, $1.7 \text{ m}^3 \text{ s}^{-1}$; b, $4.0 \text{ m}^3 \text{ s}^{-1}$. Dashed channel section represents that at the previous measurement section, $\sim 4 \text{ m}$ upstream.

bend apices are reported here. However, these sections do typify the different patterns found in the two bends. The long and cross-stream velocities were resolved into primary and secondary components using the method based on three-dimensional continuity recommended by Dietrich and Smith⁹. The results are shown in Figs 1, 2.

At both sections and flows the salient feature of the secondary circulation is the helical, skew-induced cell. However, close to the outer bank, a small cell of reverse rotation is clearly present, especially at bankfull flow. These cells are associated with distortion of the primary isovels and depression of the maximum primary velocity below the free surface in the outer bank region. The outer bank cell occupies the channel to a distance 1–2 times the bank height away from the outer bank, as observed elsewhere^{5,9,10} and predicted theoretically¹³.

The pattern of primary and secondary flows in the outer half of the channel changes little with stage at a bend, and is similar at both apices. This robustness is not present in the flow pattern in the inner half of the channel, where the two bends react differently to changing stage.

At intermediate flow, secondary flow over the point bar is directed radially outwards over the whole depth, as predicted and observed by Dietrich and Smith and by Bridge^{9,10}. The reason for this is the dominance of the centrifugal force acting outwards over the hydrostatic pressure gradient, acting inwards. Also, the flow is shelving and narrowing in the longstream direction at the inner bank, as indicated in Figs 1a and 2a by the dashed lines showing the cross-section just upstream from the bend apex. Consequently, water is driven radially outwards to maintain continuity, leading to outward secondary flow. These

arguments agree with the explanation put forward by the previous researchers^{9,10}.

At bankfull stage, the two bends show different flow patterns in the inner half of the channel. At the upstream bend, outward flow has been replaced by helical flow over most of the width, as predicted by Bridge¹⁰. The point bar has been scoured and reprofiled by the high flow and this, combined with the increased stage at almost constant width, has significantly increased the importance of the cross stream hydrostatic pressure gradient, which is now able to drive bed flow inwards. This is not the case at the downstream bend. Here, outward flow over the point bar persists up to bankfull discharge. The reason for this is that the width increases markedly with stage at the downstream bend, so that both the depth and the hydrostatic pressure gradient over the point bar remain small (Fig. 2b). Also, the point bar is more prominent in the downstream bend, so that shelving in the downstream direction is maintained at bankfull flow (Fig. 2b). This is not the case at the upstream bend, where high flow scouring produces deepening downstream at the inner bank (Fig. 1b).

We conclude that both patterns of flow in the inner half of the channel are possible and do occur in nature, depending on the morphology of the channel cross-section. Of particular importance is the stage-width relationship. Where there is marked widening with stage, outward flow persists to bankfull flow, but where width is almost constant with stage, helical flow expands almost to the inner bank. A quick survey of the Fall River bends revealed about equal numbers of bends falling into each category. As all the bends have the same bankfull discharge, sediment load, and bed and bank materials, there seems to be no obvious reason for the different morphologies, raising the possibility of there being two stable bend sections for a given set of independent controls. Clearly, point bar-flow interaction is a topic deserving further consideration and study.

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Enhancement of marine primary production by nitrogen-enriched acid rain

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The effect of acid rain on the pH of freshwater habitats is well known¹. The acidity of rainwater is largely due to hydrated oxides of sulphur, nitrogen and carbon², which also constitute nutrients essential for plant growth. Most freshwater and marine environments contain sufficient quantities of inorganic carbon and sulphur to satisfy plant growth needs, but nitrogen inputs can at times fall short of growth demands^{3,4}. Nitrogen limitation of phytoplankton growth is widespread and chronic in some marine waters^{5–7}. I

report here that nitrogen-limited conditions in North Carolina near-surface waters are ameliorated following rainfall inputs; the most acidic rainfall events led to the greatest stimulation of phytoplankton growth. Continentally-derived acid rain consistently contained more inorganic nitrogen (NO_3^- , NO_2^- and NH_4^+) than near neutral rainfall generated from oceanic fronts. Marine primary production can thus be influenced by the source of rainfall in marginal environments.

Nutrient limitation and impacts of rainfall on phytoplankton growth were examined at three Atlantic Ocean locations on the east coast of North Carolina. Bogue Sound, a euhaline (30–34 parts per 10^3 (p.p.t)) sound separating the mainland from a barrier island (Bogue Island) served as an inshore location. Continental slope waters ~1 km offshore from Bogue Banks provided a near-shore habitat, while Gulf Stream waters 25 km offshore represented a more pelagic habitat. All three locations commonly receive both acidic (continental origin) and near-neutral (oceanic origin) rainfall. Continental rain pH characteristically ranged from pH 3.5 to 5.5, while oceanic rain ranged from pH 5.5 to 6.8. Surface waters from these locations were collected in 20-l pre-cleaned polyethylene carboys throughout 1983–84, immediately brought to the Institute of Marine Sciences (IMS) and analysed for the soluble nutrients $\text{NO}_3^- + \text{NO}_2^-$, $\text{NH}_3/\text{NH}_4^+$, PO_4^{3-} and chlorophyll *a* content. Samples were collected during periods of minimal riverine runoff (salinities of at least 33 p.p.t.); however, Bogue Sound was occasionally sampled after freshwater runoff episodes, as experienced after the landfall of hurricane Dianna during mid-September 1984 (salinity = 27 p.p.t.).

Rainwater was routinely collected on the rooftop of IMS using pre-cleaned polyethylene buckets. Rainfall amounts and pH were recorded and nutrient chemistry was performed on rainwater used in bioassays.

Bioassays were performed in triplicate in 1-l pre-cleaned polyethylene Cubitainers, which were 85% transparent to photosynthetically active radiation (PAR = 400–700 nm). They were incubated in natural surface light and temperature conditions in a saltwater pond at IMS. Incubation periods lasted between 1 and 7 days. Freshly collected seawater was dispensed, followed by additions of either 10 or 20% v/v rainwater (RW) or distilled deionized water (DDW). Total bioassay volume was 900 cm^3 . Parallel N- NO_3^- (100 and 200 parts per 10^9 (p.p.b.), P- PO_4^{3-} (50 and 100 p.p.b.), and combined $\text{NO}_3^-/\text{PO}_4^{3-}$ additions were also made. A 10- μCi aliquot of ^{14}C - NaHCO_3 (68 μCi μmol ICN Corporation) was also added to each Cubitainer to monitor photosynthetic ^{14}C assimilation as a growth-response parameter. All ^{14}C assimilation results were corrected for abiotic ^{14}C precipitation and liquid scintillation quenching. The fluorometric determination of chlorophyll *a* in 90% v/v MgCO_3 -buffered acetone extracts served as an additional phytoplankton growth-response parameter.

Both oceanic and continental rain significantly ($P < 0.001$) stimulated phytoplankton primary production as observed by both ^{14}C fixation and chlorophyll *a* production (Fig. 1). Stimulation was evident with both 10 and 20% v/v additions. The more acidic continental rainfall led to much higher magnitudes of stimulation (Fig. 1). Typically, 30–70% higher phytoplankton growth yields were observed in response to acidic as opposed to near-neutral rainfall additions. Parallel NO_3^- and PO_4^{3-} additions consistently revealed exclusive NO_3^- stimulation of phytoplankton production during 1983–84. Acidic rain also led to more sustained periods of stimulation. Generally, enhanced (over control) phytoplankton growth, as monitored by chlorophyll *a* production, continued for 6–7 days in response to acid rain additions (Fig. 2). Near-neutral rainfall led to initial growth stimulation, which decreased after 2–3 days (Fig. 2). Differential growth stimulation was not directly attributable to pH; neutralized (with NaOH) acid rain yielded results identical to untreated acid rain. Similarly, near-neutral rain made more acidic did not reveal greater stimulation. Furthermore, the addition of either acid or neutral rain failed to alter ($P < 0.001$) the pre-existing pH of seawater significantly (pH = 8.36). Accord-

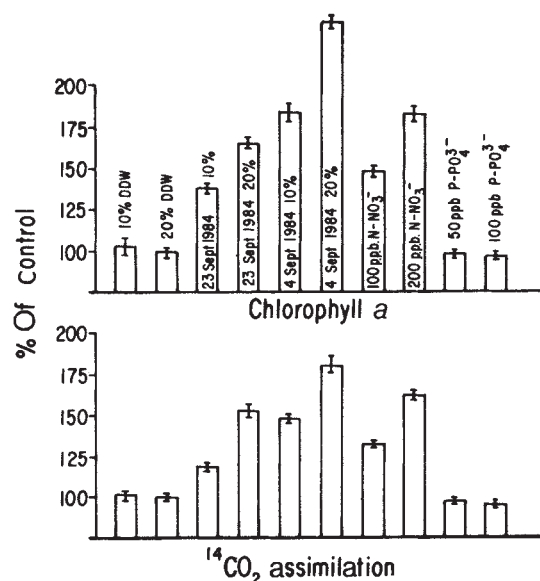


Fig. 1 Impacts of rainwater on Bogue Sound chlorophyll *a* production, and ^{14}C CO_2 assimilation following a 5-day *in situ* Cubitainer bioassay. All treatments were compared with controls (no additions). Both 10% and 20% v/v additions are shown. Rainwater collected on 4 September 1984 had a pH of 4.05, while 23 September 1984 rainwater had a pH of 5.85. Parallel 10% and 20% v/v distilled deionized water (DDW) additions revealed the impacts of equi-volume nutrient-free freshwater dilutions on Bogue Sound Water. Parallel N- NO_3^- (100 and 200 p.p.b.) and P- PO_4^{3-} (50 and 100 p.p.b.) additions consistently showed exclusive stimulation of phytoplankton growth by nitrogen. Error bars indicate variability among triplicate samples for each treatment. Biostimulation by all rainwater and N- NO_3^- additions was significant ($P < 0.001$) among the two growth parameters.

ingly, I attribute differential stimulation among, as well as between, treatments and controls to contrasting levels of dissolved inorganic nitrogen enrichment.

Chemical analyses of sea water from all sampling locations and bioassays demonstrated: (1) consistent NO_3^- depletion but PO_4^{3-} sufficiency during the main phytoplankton growth period (April–October); (2) rapid depletion of NO_3^- and $\text{NH}_3/\text{NH}_4^+$ enrichments supplied in rainwater, but a lack of concurrent PO_4^{3-} depletion either supplied in rainwater or as an individual nutrient, and (3) good agreement between the initial amount of NO_3^- , $\text{NH}_3/\text{NH}_4^+$ present in rainwater and the duration of phytoplankton growth stimulation. Lastly, a positive correlation existed between $\text{NO}_3^- + \text{NO}_2^-$ content and acidity of rainfall samples collected ($r^2 = 0.87$; $n = 15$).

Five additional bioassays, complementing those shown in Figs 1, 2, revealed that phytoplankton growth was nitrogen limited throughout the spring and summer of 1984. Atomic ratios of dissolved inorganic N : P ranged from 1 to 5 (weighted mean of 2.8), further evidence for consistent nitrogen-depleted conditions of these waters.

The choice of 10–20% (v/v) rainwater additions was based on the impacts of rainwater dilutions on near-surface (0–5 m depth) salinities following large (1–5 cm) rainfall events in coastal waters, before the dilution observed by enhanced terrigenous freshwater runoff. Near-surface salinities were commonly depressed by 2–3 p.p.t. immediately after such rainfall events. A 10% v/v bioassay rainwater addition depressed the salinity of coastal water by ~2.2 p.p.t. Hence, such additions provide reasonable approximations of natural rainwater dilution effects on near-surface seawater. Large storm systems are likely to mix rainfall inputs to depths >5 m; therefore, the estimates presented here apply to more localized short-lived storm activities, including the numerous summer thunderstorms and rapidly dissipating winter cold fronts often observed in this area.

Periodic offshore chlorophyll *a* samplings before and after storm events in 1984, substantiate bioassay-derived conclusions,

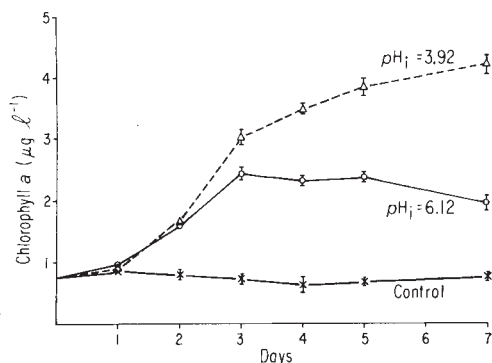


Fig. 2 Chlorophyll *a* production in Cubitainer bioassays subsampled daily following acidic versus near-neutral rainwater (10% v/v) additions. Acidic rainfall ($pH = 3.92$) characteristically showed higher magnitudes and longer-lasting stimulatory effects than near-neutral ($pH 6.12$) rainfall. Chemical analyses of acidic rainfall yielded the following results: $N-NH_4^+ = 233$ p.p.b., $N-NO_3^- + NO_2^- = 459$ p.p.b., $P-PO_4^{3-} = 9.1$ p.p.b. Near-neutral rainfall: $N-NH_4^+ = 251$ p.p.b., $N-NO_3^- + NO_2^- = 219$ p.p.b., $P-PO_4^{3-} = 3.7$ p.p.b. Control was untreated Bogue Sound water incubated in parallel. Error bars show variability among triplicate samples. Beyond day 2, all treatments were significantly ($P < 0.001$) different from each other as well as from the control.

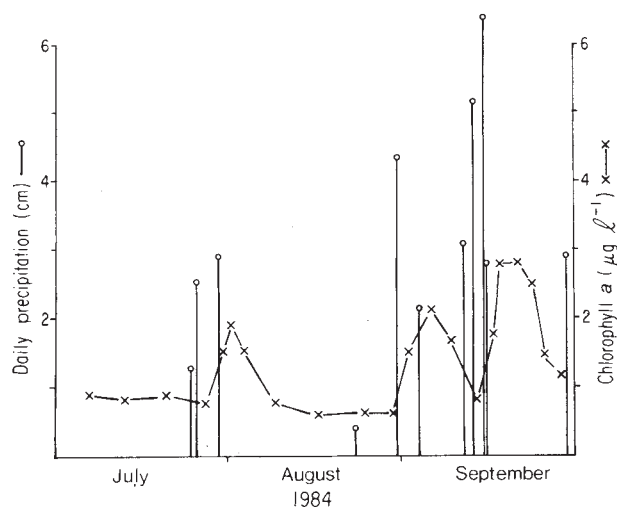


Fig. 3 Major (>0.1 cm per day) precipitation events recorded at Morehead City, North Carolina versus surface chlorophyll *a* concentrations sampled at an offshore location 5 km south-east of Bogue Banks during July, August and September 1984. All chlorophyll *a* samples were from non-turbid waters free of runoff plumes and sediment resuspension. Extensive rainfall during mid-September was due to the passage of hurricane Dianna near the North Carolina coast.

namely, that precipitation nitrogen inputs lead to significant localized enhancement of phytoplankton production in these nitrogen-depleted waters. Characteristically, a 2-day lag occurs between extensive rainfall and enhancement of near-surface chlorophyll *a* levels (Fig. 3). Chlorophyll *a* enhancement can last from a few days to several weeks. The initial (within 3 days) enhancement closely follows direct precipitation inputs, leading to small but detectable increases in NO_3^- and NH_3/NH_4^+ concentrations. Freshwater runoff sources, as delineated by suspended fine-sediment plumes and the appearance of brackish water phytoplankton species and detritus, typically do not appear in these waters until 2–3 days after the onset of chlorophyll *a* enhancement. While direct precipitation inputs seem to be responsible for initial chlorophyll *a* enhancement, terrigenous runoff probably sustains such enhancement beyond a period of several days.

Experimental and observational findings indicate that localized inorganic nitrogen enrichment attributable to direct precipitation inputs can lead to enrichment of marine primary production in near-surface waters. Annually, chlorophyll *a* concentration ranges from >1 to $5 \mu g l^{-1}$ in these waters. The observed buildup of chlorophyll *a* concentrations following direct precipitation inputs (Fig. 3) therefore constitutes a substantial fraction of maximum levels. Acidic rainfall, being enriched in NO_3^- and NO_2^- , preceded the highest magnitudes of primary production stimulation. I therefore conclude that although pH impacts of acid rain are largely insignificant in well-buffered marine habitats, nutritive impacts are both detectable and may be of long-term consequence in shaping both patterns and magnitudes of phytoplankton production. Such potential impacts are expected to be of greatest importance in waters bordering the eastern margins of land masses supporting extensive industrial and urban development. These would include the eastern seaboard of the United States and the United Kingdom, the Baltic region and the Western Pacific waters bordering Japan, Korea and China.

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Weak neutral current and β radiolysis effects on the origin of biomolecular chirality

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The possibility of a connection between the parity non-conserving weak interactions and the handedness of biomolecules has received recent attention¹. Although the asymmetric effects of the weak interactions in molecules have been estimated to be very small^{2–9}, Kondapudi and Nelson (KN)^{10–13} have shown that autocatalytic chemical systems that spontaneously break chiral symmetry^{14–19} can effectively amplify systematic chiral perturbations as small as those produced by weak neutral currents^{2–4,20}, despite the simultaneous presence of larger randomly fluctuating chiral perturbations, and hence provide a mechanism for producing the observed homochirality of biomolecules. I show here, in the light of new estimates of the asymmetric decomposition of racemic mixtures by β radiation⁹, that the chiral selection parameter due to β radiolysis can be up to six orders of magnitude larger than that resulting from weak neutral currents. This suggests that β radiolysis is more likely to be the selector of biomolecular chirality than weak neutral currents.

The same chemical kinetic model is used as in KN^{10–13} but the reactants S and T in the model are taken to be chiral, rather than achiral as assumed by KN, and only homochiral combinations of reactants with each other and with the autocatalytically formed product X are assumed to occur, for example, $S_L + T_L + X_L \rightarrow 2X_L$, $S_D + T_D + X_D \rightarrow 2X_D$. Here S and T could be, for example, chiral monomers which react to form a dimer X. Alternatively, this simple model could be interpreted as an

abbreviated approximation of a more complicated mechanism for the formation of a polymer X. Because all known biomolecules capable of catalysing their own formation (the protein/nucleic acid systems) are polymers formed from homochiral monomers, the assumption of chiral reactants rather than achiral ones seems a reasonable modification of the original KN model considered as a description of the autocatalytic formation of prebiomolecules.

The kinetic model gives the expression $dX_L/dt = k_1 S_L T_L - k_{-1} X_L + k_2 S_L T_L X_L - k_{-2} X_L^2 - k_3 X_L X_D$ for the rate of formation of X_L and a similar expression (with L and D interchanged) for formation of X_D , where S_L , T_L , and X_L denote molar concentrations of the corresponding substances. According to these equations, the enantiomers X_L and X_D are formed at different rates if the concentrations of reactants, assumed by KN¹⁰⁻¹³ and in the present work to be constant or slowly varying in time, are such that $S_L T_L$ is not equal to $S_D T_D$. The production of this inequality by asymmetric β radiolysis is considered below.

An inequality in constant reactant concentrations is equivalent to an inequality in rate constants. With the definitions $\eta = (S_L - S_D)/(S_L + S_D)$ and $S = (S_L + S_D)/2$ for reactant S, which imply $S_L = (1 + \eta)S$ and $S_D = (1 - \eta)S$, and with similar definitions for reactant T, the term $k_1 S_L T_L$ appearing in the rate equation can be written correct to first order in η as $k_1(1 + 2\eta)ST$, where it has been assumed for simplicity that η has the same value for both S and T, and where the last equation defines k_{1L} . Similarly, $k_1 S_D T_D = k_{1D}ST$ with $k_{1D} = (1 - 2\eta)k_1$. In the same way as for k_1 , the terms containing k_2 are replaced by ones containing $k_{2L} = (1 + 2\eta)k_2$ and $k_{2D} = (1 - 2\eta)k_2$. Finally, the selection parameter, defined by KN as $g = 2(k_{1L} - k_{1D})/(k_{1L} + k_{1D})$, is found to be related to η simply as $g = 4\eta$.

With the above definitions, the resulting rate equations are identical to those of the original KN model¹⁰⁻¹³, but the physical origin of the asymmetries in the effective rate constants k_1 and k_2 is quite different. In the original KN model, weak neutral currents produce inequalities $k_{1L} \neq k_{1D}$ and $k_{2L} \neq k_{2D}$ which in typical organic molecules give a resulting selection parameter $g = \Delta E/RT \sim 10^{-17}$, where ΔE is the parity nonconserving energy difference between enantiomers²⁻⁴. In spite of its smallness, KN have demonstrated that this value of g is sufficiently large to determine the eventual dominance of one of the enantiomers of X (refs 10-13). For comparison, we now consider the production of concentration inequalities $S_L \neq S_D$ and $T_L \neq T_D$ by β radiolysis⁵⁻⁹ of a racemic mixture of reactants (β radiolysis of X was also calculated and found to be less important). Here the value of η created by the asymmetric decomposition of the enantiomers depends on the strength of the β -ray source, the irradiation time, and the temperature. For organic compounds exposed to likely prebiotic sources of β radiation and temperatures below 80 °C, η has been estimated⁹ to attain values as high as 10^{-12} . It follows from the relation $g = 4\eta$ that the value of g resulting from β radiolysis can be up to five or six orders of magnitude larger than the value of g resulting from weak neutral currents. This dominance by β radiolysis is also expected for most chiral molecules containing heavy atoms, where the values of g are predicted to be larger for both types of interaction^{2-4,8}.

Hence, it is predicted that β radiolysis can produce a much larger systematic chiral perturbation than produced by weak neutral currents. If a racemic mixture of reactants is exposed for a sufficiently long period of time to sufficiently strong β radiation sources such as those which may have been present in prebiotic times⁹, and if a symmetry-breaking autocatalytic chemical reaction of the type analysed by KN¹⁰⁻¹³ then occurs, the chirality of the resulting product is more likely to be determined by β radiolysis than by weak neutral currents. Provided that an effective autocatalytic chemical reaction can be realized, an experimental test of this prediction seems possible using existing intense sources of β radiation⁹.

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Osmotic stress mimics effects of vasopressin on learned behaviour

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It has been suggested that arginine vasopressin (AVP) is involved in the retention of learned responses, in addition to its classical physiological functions of water retention and modulation of blood pressure. AVP administered subcutaneously (s.c.), intravenicularly or intracerebrally can prolong extinction of active avoidance behaviour^{1,2} and can enhance retention in inhibitory (passive) avoidance³⁻⁵. These effects have been interpreted as a direct action of AVP on the central nervous system to facilitate memory consolidation^{3,4}. AVP also has facilitatory effects on cognitive function in humans⁶, and marked deficits in AVP function have been associated with certain types of psychopathology⁷. Alternative hypotheses for the behavioural actions of AVP have involved motivational constructs such as arousal⁸, and our recent work has focused on the role of arousal resulting from the activation of peripheral visceral signals in the behavioural effects of peripherally administered AVP⁹⁻¹². The development of a specific antagonist for AVP¹³, 1-deaminopenicillamine-2-O-methyl tyrosine arginine vasopressin (dPTyr(Me)AVP), which can reverse the behavioural effects of exogenously administered AVP⁹⁻¹², has provided a powerful tool for examining the role of AVP in the behavioural responses produced by physiological challenges known to release vasopressin. However, the relationship between the behavioural effects of exogenously administered AVP and the behavioural function of endogenously released AVP has not been evaluated. We report here that a potent peripheral osmotic stimulus, the intraperitoneal (i.p.) injection of hypertonic saline, at doses known to release AVP both centrally¹⁴ and peripherally¹⁵, will produce behavioural effects similar to those of exogenously administered AVP. Furthermore, the prolongation of active avoidance induced by this osmotic stimulus is reversed by pretreatment with dPTyr(Me)AVP, suggesting that endogenously released AVP may also produce behavioural effects.

Male Wistar rats (150-220 g), each handled and weighed once previously, were trained to avoid shock in a pole jump apparatus as described previously². Briefly, during the initial training of the active avoidance response, the rats were subjected to 10 trials per day where a 5-s conditioned stimulus preceded a 0.3-0.5-mA footshock to the floor. A response during the 5-s conditioned stimulus (light illumination) was recorded as an

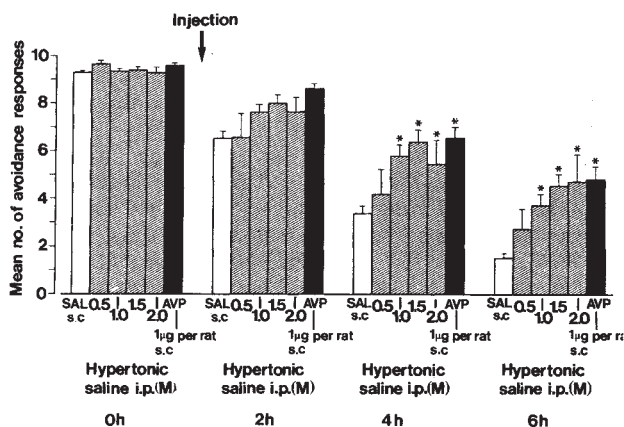


Fig. 1 Effects of different doses of hypertonic saline on extinction of active avoidance. Rats were injected with saline i.p. immediately after the first 10 extinction trials on day 4 of testing. * Significantly different from the saline group, $P < 0.05$, Student's t -test following a significant overall analysis of variance for each separate extinction session. Numbers of subjects were 83, 11, 52, 34, 11 and 42 in the saline, 0.5 M, 1.0 M, 1.5 M, 2.0 M NaCl and AVP (1 µg per rat, ~5.4 µg per kg) groups, respectively.

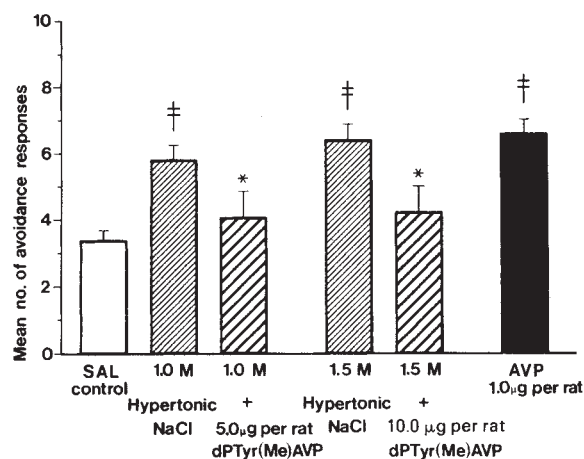


Fig. 2 Effects of the AVP antagonist on the prolongation of extinction produced by hypertonic saline. Data shown represent only those from extinction session III, but results from extinction session IV were identical. * Significantly different from hypertonic saline alone, $P < 0.05$, Student's t -test following a significant overall analysis of variance for each logical grouping; ‡ significantly different from saline, $P < 0.05$. Numbers of subjects were 83, 52, 21, 34, 10 and 42 for the saline, 1.0 M NaCl only, 1.0 M NaCl + 5.0 µg per rat (~27 µg per kg) dPTyr(Me)AVP, 1.5 M NaCl only, 1.5 M NaCl + 10 µg per rat (~54 µg per kg) dPTyr(Me)AVP, and AVP, respectively. Saline, 1.0 M NaCl, 1.5 M NaCl and AVP groups are the same as in Fig. 1 for extinction session III.

avoidance and the inter-trial interval was 40, 60 or 80 s, randomly assigned across trials. Following 3 days of acquisition, rats were subjected to a fourth day of extinction testing with all conditions identical except that the shock was turned off. Only those rats meeting the previously established acquisition/extinction criteria^{1,2} were treated; these rats all showed ≥ 14 successful avoidance responses during acquisition and ≥ 8 avoidances during the first 10 extinction trials. Only these animals were used in the experiment. This criterion was similar to that used by De Wied and associates¹ and eliminated 10% of the rats. Four blocks of 10 trials each were run; after the first 10 trials, each rat was treated with either 0.9% saline s.c., 1 µg of vasopressin dissolved in 0.9% saline, or different concentrations of hypertonic saline (0.25–2.0 M) injected i.p. in a volume of 2 ml.

Extinction testing was continued for three more blocks of 10 trials, one block every 2 h. Drinking water was available during the testing period. In a second experiment, the procedure was identical except that rats received two injections immediately after the first 10 trials of extinction: 0.9% saline or the AVP pressor antagonist was injected s.c.¹³, followed immediately by 1.0 or 1.5 M NaCl as an osmotic stimulus. All testing was done under 'blind' conditions, that is, the experimenter did not know which rats received which treatments. Each 4-day testing series consisted of 20 rats randomly assigned to the treatment groups. Each series always contained rats receiving 0.9% saline and AVP s.c., and groups receiving hypertonic saline injections combined with the antagonist were always tested with a group receiving hypertonic saline alone. As a result, many more rats were included in the 0.9% saline (control) group and the AVP group.

The hypertonic saline was injected i.p. to replicate previous studies which measured AVP levels and plasma osmolality. These doses are similar to those used by others¹⁵ to show dose-dependent increases in plasma osmolality and plasma AVP levels. In that study, a dose identical to our 1.0 M NaCl dose in rats of the same weight produced plasma osmolality of 305 mosm per kg and a plasma AVP level of 10 pg ml⁻¹. Measurements of AVP in the blood in our laboratory (S. Deyo, W. J. Shoemaker, A. Ettenberg, F.E.B. and G.F.K., unpublished results) revealed plasma levels of 13.1 ± 2.2 to 28.4 ± 3.5 pg ml⁻¹ 20 min after s.c. injection of behaviourally effective doses of AVP (0.1–1.0 µg per rat). Thus, the osmotic stimuli used here produce plasma AVP levels within the range of those produced with exogenous injection. A similar osmotic challenge (2 and 4 ml of 2 M NaCl) in anaesthetized rats provoked a dramatic increase in AVP release into a push-pull cannula localized to the lateral septum¹⁴.

Hypertonic saline injection produced a dose-dependent prolongation of active avoidance, reaching significance at doses of 1.0 and 1.5 M NaCl (Fig. 1); data from 0.25 M NaCl were not different from controls and are not shown. These results were virtually identical to those produced by 1 µg AVP injected s.c. Following the injection of hypertonic saline, the rats showed some irritability and occasionally some vocalization, but these effects disappeared within 2–3 min. Note that the rats receiving this hypertonic saline injection showed no greater ill effects than those observed following AVP. For example, exogenously administered AVP in behaviourally effective doses produces taste aversions in rats¹² and hypertonic saline at the higher doses used in these avoidance experiments (1.5 and 2.0 M NaCl) produced similar taste aversions (R.D. and G.F.K., unpublished results).

At 5 µg per rat, the vasopressin antagonist produced an attenuation of this effect of hypertonic saline (Fig. 2). A similar reversal was seen with 10 µg antagonist after treatment with 1.5 M NaCl (Fig. 2). Preliminary results indicate that this effect may be dose-dependent, in that 2.5 µg antagonist did not attenuate the prolongation of extinction produced by 1 M NaCl (mean $\pm$ s.e.m., number of avoidances for extinction session III were 5.90 ± 1.17 for 2.5 µg per rat compared with 5.79 ± 0.49 for 0 µg per rat).

These results demonstrate that an interoceptive osmotic challenge (hypertonic saline), known to release AVP, can mimic the effects of exogenous AVP on learned behaviour. Further, this behavioural effect is reversed by an AVP receptor antagonist, suggesting a causal relationship between the endogenously released AVP and the behavioural change. Similar doses of the AVP antagonists have been used to reverse the behavioural effects of exogenously administered AVP in various learning tasks^{9–10}.

It is unlikely that the antagonist effect in the present experiment is due to an action of the antagonist itself, independent of endogenously released AVP, for example by a general sedative or tranquillizing action. Except for the 100 µg dose², we have observed no effect with this antagonist in reversing prolongation of active avoidance produced by high shock levels during training. Also, the AVP antagonist did not significantly alter caffeine-stimulated locomotion at doses from 2.5 to 10 µg per rat (~17–67 µg per kg, see Table 1a). Analysis of variance of these data

Table 1 Effects of AVP antagonist dPyr(Me)AVP on caffeine-stimulated locomotor activity (a) and performance in a multiple-schedule operant conflict test (b)

		dPyr(Me)AVP dose		
		2.5 µg (17 µg per kg)	5.0 µg (33 µg per kg)	10.0 µg (67 µg per kg)
a	Saline			
	1,238 ± 103 n = 6	1,032 ± 145 n = 6	1,512 ± 283 n = 5	1,487 ± 294 n = 7
		dPyr(Me)AVP dose		
		10 µg (27 µg per kg)	20 µg (54 µg per kg)	
b	Component	Saline		
	Random interval			
	30-s schedule	108 ± 18	117 ± 18	111 ± 29
	Conflict	122 ± 30 n = 5	113 ± 19 n = 5	113 ± 22 n = 5

a, Activity was measured automatically in standard photocell activity cages in continuous 10-min epochs for 90 min following injection of dPyr(Me)AVP s.c. and 10 mg per kg caffeine (base). For details see ref. 30. b, Food-deprived rats previously trained on the conflict multiple schedule were given dPyr(Me)AVP s.c. 5 min before an 18-min test session. Schedule and testing procedure are described in ref. 16.

showed no dose effect and no dose × time interaction (both $F < 1$). The AVP antagonist also failed to produce any sedative-like actions in an operant Geller-Seifter conflict test¹⁶ that is very sensitive to minor tranquilizers. It did not alter high-rate responding maintained by the random interval 30-s schedule, and also failed to produce a release of punished responding in the conflict component (Table 1b). Finally, the antagonist did not reverse apomorphine-induced taste aversion (R.D., R. M. Bluthé, G.F.K. and M. Le M., unpublished observations). Although it is possible that the hypertonic saline produced elevations in blood pressure that contributed to the behavioural effects, NaCl-induced changes in blood pressure are also reversed by these vasopressor AVP antagonists¹⁷, indicating that such a role for pressor responses as an intervening variable with NaCl would be similar to that seen with exogenously administered AVP¹².

Finally, these and other recent data⁹⁻¹² reopen the question of the nature of brain-pituitary interactions. One unresolved problem concerns the mechanism mediating this AVP behavioural response. For example, there is little evidence that AVP itself crosses the blood-brain barrier, particularly at doses that would be liberated from the pituitary in the conditions of the present experiment¹⁸⁻²². Furthermore, central AVP pathways exist independently of the classical hypothalamo-posterior pituitary projection²³, and both central and peripheral AVP are released by the osmotic challenge used here^{14,15}. De Wied and co-workers have argued for a central site of action based on the observation that a new analogue of vasopressin (pGlu⁴, Cyt⁶AVP(4-8)), when injected peripherally, is much more active than AVP itself in facilitating passive avoidance²⁴. This analogue has no pressor activity and its effects can be reversed by an even more potent AVP pressor antagonist²⁵ injected in nanogram amounts intracerebroventricularly. Our earlier work showed that dPyr(Me)AVP can reverse the effects of centrally administered AVP²⁶, but will only reverse the behavioural effects of peripherally injected AVP when injected centrally in doses sufficient to reverse the peripheral pressor effects of the same doses^{27,28}. From this we hypothesize an effect of AVP from both central and peripheral sources acting independently but in a homologous fashion²⁹. Thus, in the present experiment, the behavioural change following osmotic challenge could result from a release of AVP from the pituitary and/or the central nervous system.

These results also have important implications for our conceptualization of the role of AVP in behaviour and of brain-body

interactions in learning. Clearly, an AVP reserve sufficient to change or even mobilize behaviour, is available in situations of extreme challenge. It will be critical to determine whether other cues can activate this system, and how this mechanism works, if at all, in normal behaviour.

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Bidirectional control of cytosolic free calcium by thyrotropin-releasing hormone in pituitary cells

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It is now established that a key step in the action of calcium-mobilizing agonists is stimulation of the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) to 1,2-diacylglycerol and inositol 1,4,5-trisphosphate (InsP₃)^{1,2}. The latter substance acts as a second messenger, controlling the release of calcium from intracellular stores (see ref. 3 for review). The bifurcating nature of the signalling system is exemplified by the fact that the other product of PtdIns(4,5)P₂ hydrolysis, 1,2-diacylglycerol, can alter cellular function by activating protein kinase C, the cellular target for several tumour-promoting agents such as the phorbol esters^{4,5}. In various tissues, including GH₃ pituitary tumour cells, a synergistic interaction between calcium ions and protein kinase C underlies agonist-induced changes in cell activity^{4,6-10}. The data presented here suggest that when GH₃ cells are stimulated by thyrotropin-releasing hormone (TRH), an agonist inducing PtdIns(4,5)P₂ hydrolysis¹¹⁻¹⁴, the two limbs of the inositol lipid signalling system interact to control free cytosolic calcium levels ([Ca²⁺]_i). At low levels of TRH receptor occupancy, [Ca²⁺]_i

increases rapidly, then declines relatively slowly. As receptor occupancy increases, the calcium signal becomes more short-lived due to the appearance of a second, inhibitory, component. This latter component, which is enhanced when $[Ca^{2+}]_i$ is elevated by high potassium depolarization, is mimicked by active phorbol esters and by bacterial phospholipase C. It seems likely that protein kinase C subserves a negative feedback role in agonist-induced calcium mobilization.

When TRH was added to GH₃ cells which had been pre-loaded with the fluorescent calcium indicator, Quin-2 (ref. 15), there was a rapid increase in fluorescence, indicative of an elevation in $[Ca^{2+}]_i$ (refs 16–20). Figure 1a shows, in confirmation of previous results²⁰, that the kinetics of the TRH-stimulated calcium signal is concentration-dependent; low concentrations of TRH (10^{-9} M) stimulate a relatively long-lived response, while higher concentrations of the peptide give a larger, more transient response. The reversal of the calcium signal with elevated TRH concentrations was more readily seen when the peptide was added following stimulation with high KCl, which has been shown to increase $[Ca^{2+}]_i$ by opening voltage-sensitive calcium channels^{16–19,21,22}. Low concentrations of TRH had no effect in these conditions, while with higher concentrations, an initial increase in fluorescence was rapidly superceded by an inhibitory component (Fig. 1b). The calcium dependence of the inhibitory mechanism is examined in the experiments summarized in Fig. 1c. Pre-stimulation with different concentrations of KCl allows $[Ca^{2+}]_i$ to be titrated to various levels. In the experi-

ment shown in Fig. 1c, the $[Ca^{2+}]_i$ values immediately before TRH addition were 110, 205 and 440 nM when the concentration of the depolarizing stimulus was 5, 20 and 40 mM KCl, respectively. Subsequent addition of TRH (10^{-7} M) led to a marked inhibitory effect only with 40 mM KCl (Fig. 1c).

There is considerable evidence that phorbol esters activate cellular processes by mimicking the effects of 1,2-diacylglycerol on protein kinase C^{5,23–25}. Addition of active phorbol esters such as 4 β -phorbol 12-myristate 13-acetate (PMA, 10^{-9} – 10^{-6} M) to GH₃ cells had no significant effect on the resting $[Ca^{2+}]_i$ (Fig. 2) or on the cellular content of InsP₃ (Fig. 2 legend). Addition of PMA (10^{-6} M) to GH₃ cells 5 min before the addition of TRH resulted in a partial inhibition of the TRH-induced rise in $[Ca^{2+}]_i$ (Fig. 2) and a reduction in the ability of the peptide to increase InsP₃ levels (Fig. 2 legend). The inhibition of both responses was especially marked when low concentrations of TRH (10^{-10} – 10^{-9} M) were used.

When PMA (5×10^{-7} M) was added to GH₃ cells previously stimulated with 40 mM KCl, $[Ca^{2+}]_i$ decreased after a lag of 30 s (Fig. 3a). This inhibitory effect, unlike that following TRH addition (Fig. 3a), was not preceded by an increase in $[Ca^{2+}]_i$. Other phorbol esters such as 4 β -phorbol 12,13-didecanoate also produced a reduction in $[Ca^{2+}]_i$ in the above conditions, whereas agents which are inactive or only weakly active in stimulating protein kinase C^{5,26–28}, such as 4 β -phorbol or 4 α -phorbol 12,13-didecanoate, were inactive at concentrations of 2×10^{-6} M (data not shown).

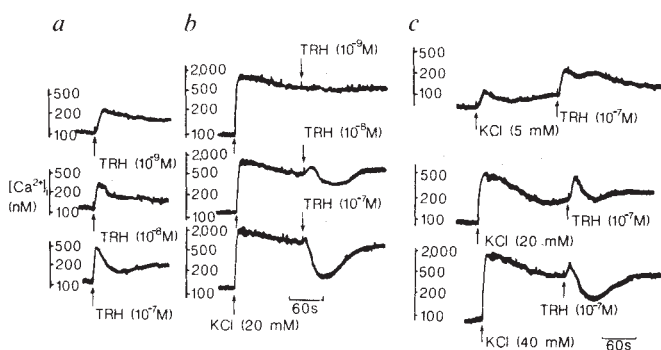


Fig. 1 Effect of TRH on cytosolic free calcium levels in untreated GH₃ cells and in GH₃ cells previously exposed to depolarizing concentrations of KCl. *a*, Response of Quin-2-loaded GH₃ cells to different TRH concentrations. Note that the decay of the calcium signal is considerably enhanced with high concentrations of the peptide. *b*, Response when TRH was added 2 min after addition of a depolarizing concentration of KCl. Addition of a similar concentration of NaCl had no effect on $[Ca^{2+}]_i$. *c*, GH₃ cells were exposed to different concentrations of KCl before the addition of TRH. Note that TRH had a marked inhibitory effect only when the highest concentration of the depolarizing stimulus was used. **Methods.** GH₃ cells were grown as described previously¹³ in Ham's F10 medium supplemented with antibiotics, horse serum and fetal calf serum. For all experiments, the cells to be used were suspended in a balanced salt solution (BSS)¹³. Cytosolic free calcium was estimated using Quin-2¹⁵. Cells ($3\text{--}5 \times 10^6 \text{ ml}^{-1}$), loaded with 2×10^{-5} M Quin-2/AM, the tetraacetoxymethyl ester of Quin-2, for 15 min at 37 °C, were washed twice in BSS and stored until use at room temperature in a darkened container. Immediately before use, the cell samples were centrifuged ($14,000 g$; 5 s) and resuspended in fresh BSS (pre-equilibrated at 37 °C) at a cell concentration of $3\text{--}5 \times 10^6 \text{ ml}^{-1}$. Fluorescence measurements were made at 37 °C on stirred samples (2 ml) of these cells using a Perkin-Elmer LS-3 spectrophotofluorimeter; excitation wavelength, 339 nm; emission wavelength, 492 nm. At the end of the analysis, 5×10^{-5} M digitonin was added to measure the maximum fluorescence ($F_{\max}$) and then excess alkaline EGTA was added to measure minimum fluorescence ($F_{\min}$). The values of $[Ca^{2+}]_i$ were calculated from the equation: $[Ca^{2+}]_i = K_D \times (F - F_{\min}) / (F_{\max} - F)$, taking a value of 115 nM as the K_D of Quin-2 for Ca^{2+} (ref. 15). Neither TRH nor any of the other drugs used in this study had any effect on the fluorescence of GH₃ cells which had not been loaded with Quin-2 (data not shown).

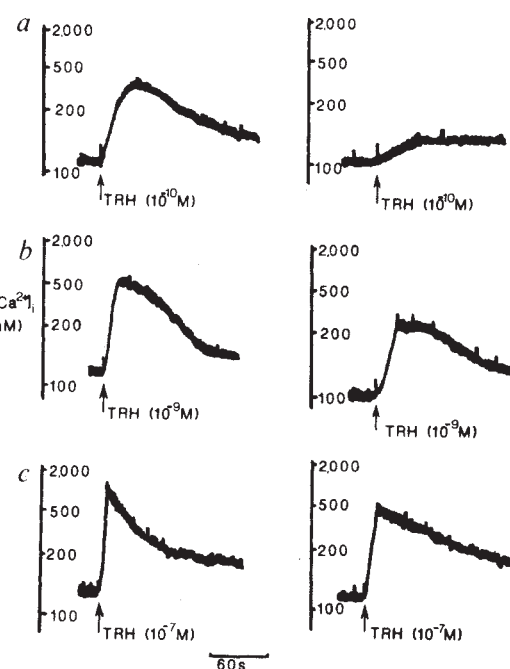


Fig. 2 Effect of 4 β -phorbol 12-myristate 13-acetate (PMA) on basal and TRH-stimulated cytosolic free calcium levels in GH₃ cells. The cytosolic free calcium concentration of Quin-2-labelled GH₃ cells was measured as described in Fig. 1 legend. The traces in *a*, *b* and *c* show the effects of preincubation of GH₃ cells for 5 min with dimethyl sulphoxide (vehicle of PMA, 0.1% (v/v) left) or PMA (10^{-6} M) (right). Dimethyl sulphoxide, at this concentration, did not alter the basal level of $[Ca^{2+}]_i$ or affect the response of the cells to TRH (data not shown). Note that although PMA did not significantly reduce basal levels of $[Ca^{2+}]_i$ in GH₃ cells, it did reduce the response (and, in particular, the rate of rise) to TRH (10^{-10} – 10^{-7} M). The GH₃ cell content of InsP₃, measured as described in Table 1 legend, was unaffected by 5 min preincubation with PMA (10^{-6} M) (4,622 $\pm$ 533 d.p.m. per sample, control; 4,517 $\pm$ 286 d.p.m. per sample, PMA-treated). This value rose within 5 s of TRH addition to 5,866 $\pm$ 177 (10^{-9} M TRH) and 9,777 $\pm$ 711 (10^{-7} M TRH) d.p.m. per sample. Corresponding values in PMA-treated cells were 5,016 $\pm$ 187 (5 s, 10^{-9} M TRH) and 6,933 $\pm$ 177 (5 s, 10^{-7} M TRH) d.p.m. per sample.

Table 1 Effect of bacterial phospholipase C on the GH₃ cell content of 1,2-diacylglycerol and inositol trisphosphate

Addition	Incubation time (s)	1,2-Diacylglycerol (d.p.m. per sample)	InsP ₃
Control	—	1,756 ± 80	1,079 ± 182
Phospholipase C (0.1 U ml ⁻¹)	180	7,756 ± 869*	1,278 ± 100
Phospholipase C (0.1 U ml ⁻¹)	360	12,624 ± 367*	1,427 ± 98
Phospholipase C (0.5 U ml ⁻¹)	30	3,188 ± 203†	1,079 ± 132
Phospholipase C (0.5 U ml ⁻¹)	180	17,800 ± 221*	1,560 ± 50
Phospholipase C (2.5 U ml ⁻¹)	30	8,824 ± 363*	1,328 ± 66
Phospholipase C (2.5 U ml ⁻¹)	180	36,450 ± 1610*	2,872 ± 282†

The GH₃ cell content of 1,2-diacylglycerol and InsP₃ was measured in cells which had been pre-labelled to isotopic equilibrium with ³H-glycerol (25 µCi per dish) or ³H-inositol (5 µCi per dish) respectively. The methods have been described previously^{13,14}. Briefly, GH₃ cell suspensions (0.5 ml), pre-labelled for 3–4 days with ³H-glycerol or ³H-inositol (Amersham International), were incubated with phospholipase C (from *Clostridium perfringens*, Sigma lot 53F-9007) for the times indicated. The reaction was stopped by the addition of 1.5 ml chloroform/methanol (1:2 v/v; for 1,2-diacylglycerol extraction) or 0.5 ml 20% (w/v) trichloroacetic acid (for InsP₃ estimation). The cell content of the two products of PtdIns(4,5)P₂ hydrolysis were then measured as outlined in refs 13 and 14. In different experiments, the samples of cell suspension contained 0.4–0.6 mg of cellular protein. The values shown are the means ± s.e.m. for three or four samples.

* $P < 0.001$; † $P < 0.01$; significantly different from the untreated control.

Addition of bacterial phospholipase C to GH₃ and various other cells stimulates 1,2-diacylglycerol formation and activates protein kinase C-dependent protein phosphorylation and cellular responses^{9,29–32}. We found that low activities of the enzyme (≤ 100 mU ml⁻¹) led to a reduction in $[Ca^{2+}]_i$ after a lag of 3 min (Fig. 3b). At this time, cellular 1,2-diacylglycerol levels increased significantly, with no change in InsP₃ content (Table 1) and, as reported elsewhere³², protein kinase C-dependent protein phosphorylation also increased. Higher enzyme activities led to more rapid increases in 1,2-diacylglycerol content in the absence of changes in cell InsP₃ levels (Table 1), and this was also associated with a decrease in $[Ca^{2+}]_i$ (Fig. 3b). When the incubation time was lengthened, however, phospholipase C (> 500 mU ml⁻¹) did elicit an elevation in InsP₃ (Table 1). As might be expected from the effects of InsP₃ on various cells³, this was associated, in the absence of depolarization, with a rise in $[Ca^{2+}]_i$ (Fig. 3c) or, in the presence of depolarization, with a temporary stabilization of $[Ca^{2+}]_i$ (Fig. 3b).

There is now strong evidence that TRH, acting on GH₃ cells, produces the initial rise in $[Ca^{2+}]_i$ by a mechanism dependent on InsP₃-stimulated release of intracellular calcium^{20,33}. Other studies show that this mechanism is fully activated at relatively low levels of receptor occupancy²⁰. The second, inhibitory, mechanism, which predominates at high levels of receptor occupancy, seems to be under the control of protein kinase C, as it can be mimicked by treatments that activate the enzyme in the conditions used. Moreover, at the time the inhibitory phase is becoming evident, 1,2-diacylglycerol levels have already been elevated by TRH^{12,13}. It is possible that the inhibitory mechanism is effected by: (1) stimulating the intracellular sequestration of calcium, (2) stimulating calcium extrusion from the cell, (3) inhibiting calcium mobilization or (4) blocking calcium entry across the plasma membrane. The latter two explanations seem unlikely, as the inhibitory effect occurs with either TRH or depolarization as the stimulus for calcium elevation; there is good evidence^{16,21} that the source of calcium differs in the two cases, with intracellular calcium the source for the

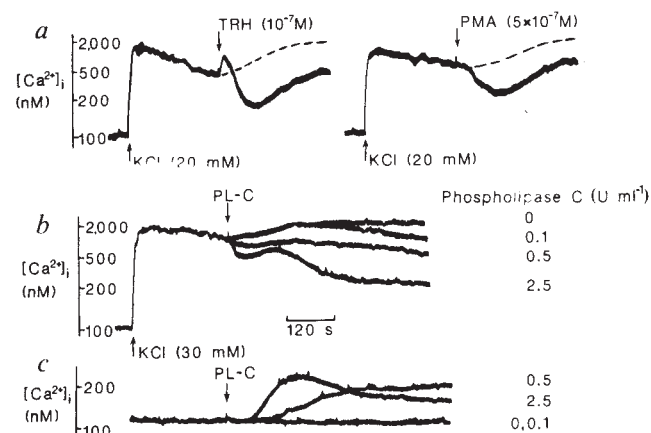


Fig. 3 Effect of agents that stimulate protein kinase C activity on cytosolic free calcium levels in GH₃ cells. The cytosolic free calcium concentration of Quin-2-labelled GH₃ cells was monitored as described in Fig. 1 legend. The traces in *a* show representative responses of GH₃ cells to the addition of TRH and PMA. The dotted line indicates the control response. Note that the phorbol ester elicited a reduction in $[Ca^{2+}]_i$ without a preceding rise. *b*, *c*, Effect of addition of bacterial phospholipase C (PL-C). The enzyme (from *C. perfringens*, Sigma lot 53F-9007) elevated $[Ca^{2+}]_i$ itself after a lag of ~60 s (panel *c*) when high activities were used. In cells whose $[Ca^{2+}]_i$ had previously been elevated with KCl, phospholipase C induced a reduction in the level of the cation (panel *b*). The onset of the response was dependent on the activity of the enzyme used. Corresponding changes in the cell content of 1,2-diacylglycerol and InsP₃ are shown in Table 1.

TRH effect and influx of extracellular calcium the mechanism by which high K⁺ elicits its effect. Indeed, the TRH-induced calcium signal still fades rapidly with high concentrations of the peptide in the presence of 2 mM EGTA (data not shown). It is evident, nevertheless, that activators of protein kinase C can inhibit the TRH-induced rise in InsP₃ and $[Ca^{2+}]_i$ in GH₃ cells and diminish calcium mobilization and phosphatidate accumulation in other cells^{34,35}. It is unlikely, however, that this effect underlies the inhibitory phase of TRH action because: (1) this phase is mimicked by phorbol esters in conditions in which no InsP₃ formation occurs (with high K⁺ as the stimulus), (2) levels of InsP₃ remain elevated in response to TRH for at least 30 min after peptide addition, although the calcium signal fades rapidly with high concentrations of TRH³⁶ and (3) such a block of InsP₃ production cannot reasonably explain why TRH is able to decrease $[Ca^{2+}]_i$ below the elevated steady-state value achieved after high K⁺ depolarization; only the increment due to TRH would be expected to be reversed by this mechanism. It seems likely that the inhibition of InsP₃ formation represents an additional mechanism which may have a general role in limiting information flow through the inositol lipid signalling system in chronically stimulated cells, rather than over the initial seconds of the agonist-induced response. The most plausible mechanism for the inhibitory phase of TRH action, as studied here, is that sequestration or extrusion of the cation is facilitated by protein kinase C. ATP-dependent calcium transport across both intra- and extracellular membranes has been reported to be sensitive to protein kinase C^{37,38}. Further work is under way to clarify this point.

Whatever the mechanism, it is evident that both elevated $[Ca^{2+}]_i$ and 1,2-diacylglycerol levels are required for the negative feedback system to operate effectively. This suggests that the mechanism by which calcium is removed must be primed with an appropriate amount of calcium before it can be modulated by protein kinase C—an elegant and economical way of buffering excessive changes in $[Ca^{2+}]_i$ after agonist stimulation. These data demonstrate the flexibility of control which is a major advantage of the bifurcating inositol lipid signalling system.

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Intracellular Ca indicator Quin-2 inhibits Ca^{2+} inflow via Na_i/Ca_o exchange in squid axon

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Until recently, intracellular free calcium has been amenable to measurement and investigation only in cells large enough to permit either microinjection of a suitable Ca sensor such as aequorin or arsenazo III or insertion of a Ca-sensitive microelectrode^{1,2}. This constraint on cell size was removed by the development of the fluorescent Ca^{2+} -sensitive dye Quin-2 and its acetoxymethyl ester, which can be introduced into a wide range of cell types^{3,4}. A major requirement of any intracellular Ca^{2+} indicator is that it should not disturb intracellular Ca^{2+} homeostasis and Quin-2 is generally considered to be satisfactory in this respect⁵. We now report that injection of Quin-2 into squid (*Loligo forbesi*) axons can almost completely abolish one component of Ca^{2+} entry—intracellular Na^+ (Na_i)-dependent Ca^{2+} inflow, which occurs via Na/Ca exchange⁶. Mixtures of Ca and Quin-2 that buffer an ionized Ca^{2+} at close to physiological concentrations also block Na_i -dependent Ca^{2+} inflow but these same mixtures fail to block the extracellular Na^+ (Na_o)-dependent extrusion of Ca^{2+} , showing that Quin-2 acts specifically on Ca^{2+} inflow.

Depending on the size and direction of the electrochemical gradient for Na^+ , the Na/Ca exchange system can effect net movement of Ca^{2+} either into or out of the cell. For squid axons the exchange fluxes are most easily monitored either as the Ca_o -dependent ^{22}Na efflux (Ca_o/Na_i exchange mode) or the Na_o -dependent ^{45}Ca efflux (Na_o/Ca_i exchange mode)². Because external Na^+ and Ca^{2+} compete for the exchanger, Ca_o -dependent ^{22}Na efflux is most conspicuous in Na^+ -loaded axons immersed in Na^+ -free media⁶, although a small component is detectable in physiological conditions. Figure 1 shows an experiment in which both the Ca_o -dependent ^{22}Na efflux (Ca_o/Na_i exchange) and the K_o -dependent ^{22}Na efflux (Na/K exchange pump) were monitored before and after injection of Quin-2 to give a final axoplasmic Quin-2 concentration of $\sim 300 \mu\text{M}$. Within 2 min of injecting the Quin-2, the Ca_o -dependent ^{22}Na efflux started to fall and after 10 min $<10\%$ of the initial Ca_o -dependent ^{22}Na efflux was detectable. Subsequent examination of the K_o -dependent ^{22}Na efflux revealed that the Na/K pump was essentially unaltered, showing that Quin-2 selectively inhibits the Ca_o/Na_i exchange flux. Essentially similar results were obtained with final axoplasmic Quin-2 concentrations as low as $150 \mu\text{M}$. Inhibition by Quin-2 is not confined to the Ca_o -dependent ^{22}Na efflux: the associated Ca^{2+} inflow is also

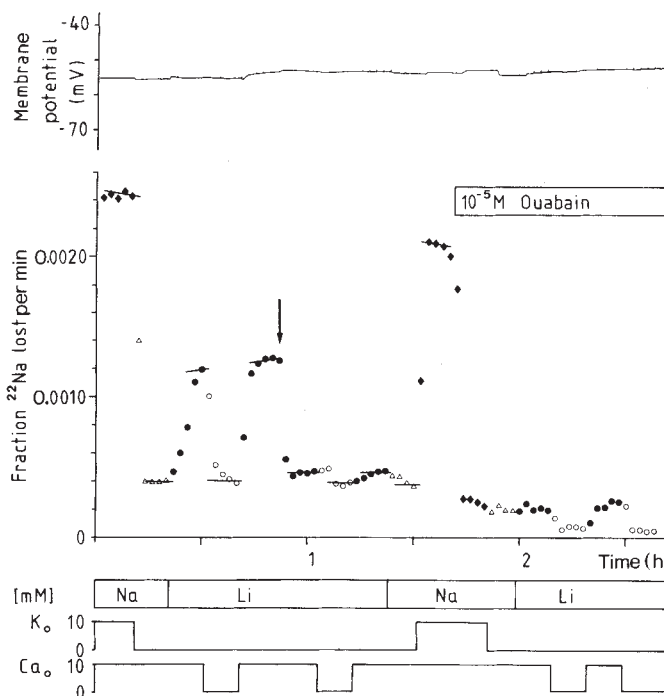


Fig. 1 Effect of Quin-2 injection on the various components of the ^{22}Na efflux from an axon of *Loligo forbesi*. The magnitude of the sodium pump flux was first determined as the reduction of the ^{22}Na efflux brought about by removing external K^+ . In the absence of K^+ , subsequent iso-osmotic replacement of NaCl by LiCl activates the ^{22}Na efflux and the whole of this activated flux is dependent on external Ca^{2+} . Injection of Quin-2 selectively inhibits the Ca_o -dependent ^{22}Na efflux, because subsequent re-introduction of potassium still activates a large ouabain-sensitive ^{22}Na efflux. The different symbols reflect solution changes, as indicated at the bottom.

Methods. After dissecting away the surrounding small nerve fibres, the giant axon was cannulated and injected with a small patch of ^{22}Na . The injector was removed, loaded with 10 mM Quin-2 (tetrapotassium salt; Calbiochem) in 100 mM MOPS buffer, pH 7.4 and reinserted. Sampling of ^{22}Na in the superfusate began 65 min after injection of isotope. The membrane potential was monitored continuously by means of an axial intracellular microelectrode. At the time indicated by the arrow, Quin-2 was injected so that the column of Quin-2 overlapped the region of axon containing ^{22}Na . The artificial sea water contained 400 mM NaCl , 100 mM MgCl_2 , 2.5 mM NaHCO_3 , pH 7.8 with potassium and calcium as indicated. Periods for which NaCl was replaced iso-osmotically by LiCl are indicated at the bottom, and the membrane potential (uncorrected for any junction potential) at the top. The axon was from a squid killed at sea and brought to the laboratory in refrigerated sea water; this results in appreciable Na^+ -loading of the axon, which increases the size of the Ca_o/Na_i exchange flux⁶. Axon diameter $880 \mu\text{m}$. Temperature 17°C .

inhibited. Thus the Ca^{2+} influx into control axons preinjected with morpholinopropanesulphonic acid (MOPS; 3.5 mM final concentration, pH 7.4) and subsequently immersed in Li-seawater was reduced to 25% of its initial value after injection of the same buffer containing Quin-2 to give an axoplasmic Quin-2 concentration of $\sim 350 \mu\text{M}$. As Na_i/Ca_o exchange is only one component of the measured Ca^{2+} influx, this may reflect a larger inhibition of the Na_i/Ca_o exchange moiety.

It has been known for some years that injection of EGTA into axons can inhibit Ca_o -dependent ^{22}Na efflux and the associated Ca^{2+} influx^{7,8}, and the data of Fig. 1 shows that the chemically related Quin-2 closely resembles EGTA in this respect. Both Quin-2 and EGTA are still effective inhibitors when injected with sufficient calcium to give a buffered free Ca^{2+} concentration close to the physiological value of 100 nM. Tests with other chelators revealed that injection of BAPTA (bis(*O*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid), EDTA or NTA (nitrilotriacetic acid) to give a final axoplasmic concentration in the range 0.5–1.0 mM also inhibits selectively the Ca_o/Na_i exchange. In the same concentration range, arsenazo III exhibits a transient inhibition only. Of particular interest is the finding that injection of the chelator 1,2-dihydroxybenzene-3,5-disulphonate (Tiron); 1 mM final concentration, which has a very low affinity for Ca^{2+} but fairly high affinity for many heavy metal divalent cations⁹, fails to block Ca_o/Na_i exchange. Although this result suggests that those chelators which block do so by stabilizing free Ca^{2+} and not by removing another essential divalent cation¹⁰, other possibilities are not completely excluded.

While the present experiments demonstrate that calcium-chelators can block Na^+ and Ca^{2+} movements via Ca_o/Na_i exchange, the same chelators have little or no effect on Na_o/Ca_i exchange. Thus, injection of a mixture of 50 mM Quin-2 and 25 mM CaCl_2 , which buffers the free Ca^{2+} close to the physiological concentration, does not alter the properties of the Na_o -

dependent ^{45}Ca efflux (Na_o/Ca_i exchange). Essentially similar results are seen after injection of Ca^{2+} -EGTA mixtures. It would seem that Ca_o/Na_i exchange is particularly vulnerable to inhibition by intracellular chelators.

Experiments on dialysed axons provide a possible explanation for these findings¹¹. Ca_o/Na_i exchange requires micromolar amounts of intracellular free Ca^{2+} for activation. It is unknown why Ca_i is needed to promote Ca^{2+} inflow but it seems to constitute a form of positive feedback which can be disrupted by the presence within the cell of a strong chelator².

These findings have two important implications: (1) experiments on Quin-2-loaded cells may fail to reveal Ca^{2+} inflow via Ca_o/Na_i exchange, especially when the Quin-2 level in the cell is $\geq 150 \mu\text{M}$, which is quite common in Quin-2 studies. (2) Loading cells with millimolar amounts of Quin-2 (as the acetoxymethyl ester) might provide a relatively simple means of effecting selective inhibition of the Ca_o/Na_i exchange mode of the Na/Ca exchanger; it might, for example, be a useful way of examining the contribution of the Ca_o/Na_i exchange system, which is known to be voltage-sensitive¹², to ionic currents in tissues such as the heart.

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Expression of a mammalian Na–H exchanger in muscle fibres of the giant barnacle

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It is now well established that the internal pH (pH_i) of mammalian cells is regulated by means of a plasma membrane transport system that exchanges extracellular Na^+ for intracellular H^+ (ref. 1). Furthermore, modulation of the activity of the Na–H exchanger seems to have a crucial role in the action of various mitogens and growth factors^{2–4}. The possibility that such a mammalian Na–H exchanger might be efficiently expressed in a giant invertebrate cell was suggested to us by recent results of Barnard⁵ and Miledi and colleagues^{6,7}, who demonstrated in frog oocytes the expression of various plasma membrane channels that presumably were encoded by the mammalian messenger RNA with which the oocytes had been injected. We used muscle fibres of the giant barnacle, which normally have no demonstrable Na–H exchanger activity⁸, and report here that, when injected with poly(A)⁺ RNA isolated from rabbit liver, the muscle fibres express a Na–H exchanger. No such expression is observed, however, when the injected material is pretreated with ribonuclease A. As hepatocytes are known to possess a Na–H exchanger⁹, the most straightforward interpretation of our data is that a mammalian Na–H exchanger has been expressed in the muscle fibre of an invertebrate.

Muscle fibres of the giant barnacle (*Balanus nubilis*) were isolated as described previously¹⁰, with one end of the fibre attached to the shell and the opposite (tendon) end free. The tendon of an individual fibre was cannulated, and through this cannula a glass injection pipette ($\sim 125 \mu\text{m}$ diameter) was advanced about 3 cm into the muscle, along the fibre's longitudinal axis. As the injection pipette was withdrawn from the fibre, fluid was deposited in the cell at the rate of $\sim 1 \mu\text{l per cm}$ of fibre length. Fibre diameter was $\sim 1 \text{ mm}$. In the first series of experiments, the injected material contained $\sim 1 \mu\text{g } \mu\text{l}^{-1}$ of poly(A)⁺ RNA (for a total dose of 3 μg RNA per fibre), isolated from rabbit liver as described in Fig. 1 legend. After the injection, the muscle fibre was incubated in artificial sea water (ASW) at 16 °C for $\sim 18 \text{ h}$. Immediately before the assay for Na–H exchange, the fibre was soaked for $\sim 15 \text{ min}$ in Ca-free ASW, and then severed at the tendon and shell ends. The central portion of the fibre, $\sim 2.5 \text{ cm}$ in length, was transferred to a standard internal-dialysis chamber, cannulated at both ends. A length of dialysis tubing was inserted through one cannula, down the length of the fibre, and out of the opposite cannula. By perfusing this tubing with dialysis fluid of known composition, we were able to achieve good control over the intracellular environment¹¹. A pH-sensitive microelectrode of the design of Hinkle¹² was inserted through one cannula, and its tip advanced to the centre of the fibre. An open-tipped reference electrode filled with 3 M KCl was advanced similarly through the opposite cannula, permitting the continuous monitoring of pH_i , which was plotted on a strip-chart recorder. The actual assays for Na–H exchanger activity were performed in Ca-containing ASW.

Our strategy for detecting a mammalian Na–H exchanger in barnacle muscle was to block selectively the fibre's normal pH_i -regulating system. Regulation of pH_i in this cell^{10,13,14}, as well as in giant cells from molluscs^{15,16}, is mediated by a plasma membrane transport system that seems to exchange external

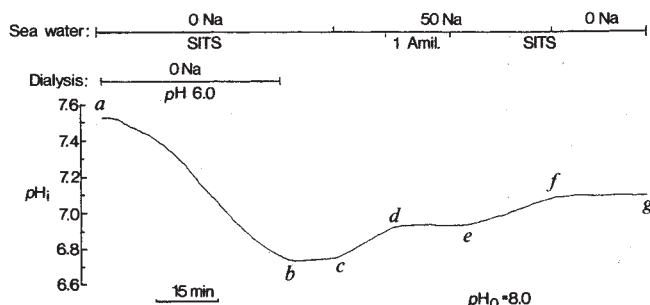


Fig. 1 pH_i recovery in a barnacle muscle fibre injected with poly(A)⁺ RNA. See text for a detailed explanation of the experiment. The pH 7.4 dialysis fluid (DF) had the following composition (in mM): 243 K⁺, 7 Mg²⁺, 8 Tris⁺, 34 Cl⁻, 175 glutamate, 2 EGTA, 4 ATP, 44 of the anionic form of HEPES, 56 of the neutral form of HEPES, 348 mannitol and 0.5 phenol red. The osmolality was adjusted to ~975 mosm per kg with mannitol or water, and the pH to 7.4 with KOH or HEPES. The pH 6.0 or 6.5 DF had a similar composition, except that the buffer was 100 mM 2-(*N*-morpholino)ethanesulphonic acid (MES). The 0-Na ASW had the following composition (in mM): 440 *N*-methyl-D-glucammonium (NMDG⁺), 10 K⁺, 11 Ca²⁺, 44 Mg²⁺, 55 Cl⁻, 5 of the anionic form of *N*-2-hydroxyethylpiperazine-*N*-3-propanesulphonic acid (EPPS), and 5 of the neutral form of EPPS. The pH was 8.0 and the osmolality ~975 mosm per kg. In the 50-Na or 440-Na ASW, NMDG⁺ was replaced with Na⁺ on a mole-for-mole basis. 0.5 mM SITS or 1 mM amiloride were added as powders immediately before use. The poly(A)⁺ RNA was prepared as follows. Fresh rabbit liver was minced in cold phosphate-buffered saline and then the tissue was homogenized in a Waring blender. SDS was added to a final concentration of 0.2%, and the tissue was disrupted in a Dounce homogenizer with the loose-fitting pestle. The volume was increased to about 10 times that of the original tissue by the addition of a solution containing 100 mM NaCl, 10 mM Tris-HCl and 5 mM EDTA (pH 7.5). The suspension was extracted three times with water-saturated phenol. The combined interfaces from the phenol extractions were extracted once with 100 mM Tris-HCl (pH 9.0) to recover polyadenylated RNA which might have partitioned into the interface. This high-pH extract was combined with the original aqueous phase, and the nucleic acids were precipitated overnight after the addition of 0.3 M sodium acetate (pH 6.0) and 2.5 vols of ethanol. The precipitate was dissolved in 10 mM Tris-HCl (pH 7.5) and the RNA was selectively precipitated at -20 °C overnight with 3 M NaCl. The precipitate was washed three times with 3 M sodium acetate (pH 6.0) and once with 90% ethanol, and then dried. The crude RNA was dissolved in distilled water and fractionated by chromatography on oligo(dT)-cellulose, as described previously¹⁸, to isolate the poly(A)⁺ RNA. The peak fractions from the column were precipitated with ethanol, dried and resuspended either in pH 7.4 DF or a solution containing 2 mM EGTA, 100 mM HEPES and 0.5 mM phenol red (pH ~7.5, osmolality ~975 mosm per kg). The final concentration of poly(A)⁺ RNA was 1 µg µl⁻¹ in the six experiments similar to that shown here and 0, 0.1 or 0.32 µg µl⁻¹ in the 11 additional experiments of the dose-response study.

Na⁺ and HCO₃⁻ for internal Cl⁻ and H⁺. This Na/HCO₃-Cl/H exchanger is functionally quite distinct from mammalian Na-H exchangers⁸. The Na/HCO₃-Cl/H exchanger has an absolute requirement for Na⁺, HCO₃⁻ and Cl⁻, is blocked by disulphonic stilbenes such as 4-acetamido-4'-isothiocyanostilbene-2,2'-disulphonate (SITS), and is unaffected by the diuretic amiloride. Na-H exchangers, on the other hand, require Na⁺ (but not Cl⁻ or HCO₃⁻), are inhibited by amiloride and are insensitive to SITS.

Figure 1 shows the result of an experiment on a muscle fibre that had been injected with 3 µg poly(A)⁺ RNA ~18 h previously. An *in vitro* translation assay had previously shown that this RNA sample stimulated protein synthesis. The fibre was pretreated for 23 min by dialysing it with a Na-free fluid at pH 7.4. During this time, the fibre was also exposed to a nominally Na⁺- and HCO₃⁻-free ASW containing 0.5 mM SITS. As the barnacle muscle's own pH_i -regulating mechanism requires HCO₃⁻ and is blocked by SITS¹⁰, our treatment should

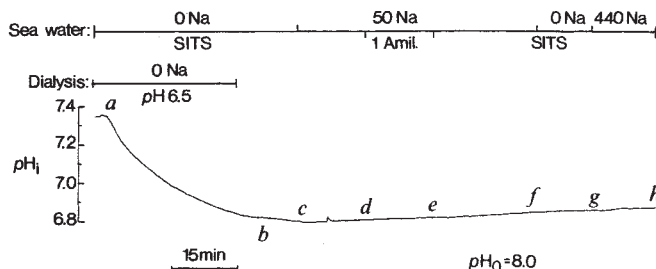


Fig. 2 pH_i recovery in a barnacle muscle fibre injected with fluid pretreated with ribonuclease. See text for details of the experiment. Poly(A)⁺ RNA was isolated from rabbit liver, as outlined in Fig. 1 legend. We verified that this sample of RNA, when injected into a muscle fibre, caused the expression of a Na-H exchanger. The remainder of the poly(A)⁺ RNA was incubated with ribonuclease A (200 µg ml⁻¹) at 21 °C for 2 h in a solution buffered to pH 8.5 with Tris. The solution was extracted first with phenol and then with chloroform/butanol. Sodium acetate was added to the aqueous phase to a concentration of 0.3 M (pH 6). After the addition of 2.5 vols of ethanol, the mixture was incubated for 2 h at -20 °C, and then centrifuged. The precipitate was resuspended at a concentration equivalent to 1 µg of original poly(A)⁺ RNA per µl.

have prevented any contribution of the barnacle's Na/HCO₃-Cl/H exchanger to pH_i . At point *a* in Fig. 1, when the pH of the dialysis fluid was lowered to 6.0, pH_i slowly fell. Dialysis was halted as pH_i reached ~6.75, returning control of pH_i to the fibre (point *b*). In the absence of external Na⁺, pH_i drifted slowly upwards (points *b*, *c*). The addition of 50 mM Na⁺ to the ASW caused pH_i to increase much more rapidly (points *c*, *d*); this pH_i recovery, which is typical of an active acid-extruding mechanism, was completely blocked by 1 mM amiloride (points *d*, *e*), a known inhibitor of Na-H exchange¹⁷. Furthermore, removal of amiloride allowed the pH_i recovery to continue (points *e*, *f*). Finally, the removal of external Na⁺ blocked further recovery (points *f*, *g*).

The Na-dependent, amiloride-sensitive pH_i recovery observed in this experiment is indicative of Na-H exchange. In 17 similar experiments, we studied expressed Na-H exchanger activity at injected poly(A)⁺ RNA doses of 0-3 µg per fibre, and fitted the data to the Michaelis-Menten equation using a nonlinear least-squares method. The apparent maximal pH_i recovery rate was 0.66 pH units per h, with the half-maximal rate occurring at a dose of 0.6 µg per fibre. In two experiments with the potent amiloride analogue 5'-(*N*-methyl-*N*-isopropyl)-amiloride (not shown), 10 µM of the drug inhibited the Na-dependent pH_i recovery by an average of 71%. Na-H exchanger activity was also found in barnacle muscle fibres injected with 0.95 µg per fibre of poly(A)⁺ RNA isolated from rat liver. The average pH_i recovery rate in two experiments was 0.40 pH units per h, comparable to that observed for fibres injected with the same dose of rabbit RNA. In one such experiment, the recovery was blocked by 1 mM amiloride, and inhibited 74% by 10 µM 5'-(*N*-methyl-*N*-isopropyl)-amiloride.

To determine whether it was the injected mRNA, rather than some other substance, that was responsible for the expression of the Na-H exchanger, we performed six experiments in which the injected material was treated with ribonuclease A in order to degrade the mRNA (see Fig. 2 legend for details). After the muscle fibre had been acid-loaded, the addition of 50 mM Na⁺ to the ASW produced little if any increase in pH_i (Fig. 2, points *c*-*f*). Amiloride produced no detectable inhibition (points *d*, *e*). Even the addition of 440 mM Na⁺ to the ASW (points *g*, *h*) failed to elicit a sizeable pH_i recovery. In six such experiments, the mean Na-dependent pH_i recovery rate was 0.05 ± 0.08 pH units per h, which is not significantly different from zero ($P > 0.35$). Thus, the expression of a Na-H exchanger requires the injection of a ribonuclease-sensitive substance, presumably mRNA.

The RNA we injected is a mixture of mRNA for many different proteins. Until the mRNA responsible for the expression of the Na-H exchanger is isolated, it will be impossible to determine whether the injected RNA caused the expression of a native but previously unexpressed Na-H exchanger, or whether the injected RNA directly encoded a Na-H exchanger. If the latter explanation is correct, this would be the first example of the expression of a mammalian ion exchanger or co-transporter in a foreign system. Our approach for expressing a Na-H exchanger could have important implications for studying the properties and regulation of Na-H exchange, and suggests an approach for cloning the gene(s) for the Na-H exchanger.

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Structural organization of the *bcr* gene and its role in the Ph' translocation

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The Philadelphia (Ph') chromosome, an abnormal chromosome 22 (ref. 1), is one of the best-known examples of a specific human chromosomal abnormality strongly associated with one form of human leukaemia, chronic myelocytic leukaemia (CML). The finding² that a small region of chromosome 9 which includes the *c-abl* oncogene is translocated to chromosome 22 prompted studies to elucidate the molecular mechanisms involved in this disease. We have demonstrated previously that the chromosome 9 of one patient with CML contains a breakpoint 14 kilobases (kb) 5' of the most 5' *v-abl*-homologous exon³. These data suggest a role for *c-abl* in CML, a theory supported by the presence of an abnormally sized *abl* messenger RNA^{4,5} and protein⁶ in the CML cell line K562. The region involved in the translocation on chromosome 22 has also been identified: all Ph'-positive patients examined to date have a breakpoint within a 5.8-kb region, for which we have proposed the name 'breakpoint cluster region' (*bcr*)⁷. To determine whether *bcr* contains protein-encoding regions, probes from *bcr* were tested for their ability to hybridize to complementary DNA sequences. A 0.6-kb *HindIII/BamHI* *bcr* restriction enzyme fragment proved suitable for isolating several cDNA clones from a human fibroblast cDNA library⁸. Using *bcr* cDNA sequences, we obtained data strongly suggesting the presence of a chimaeric

bcr/abl mRNA in the leukaemic cells of Ph'-positive CML patients. The recent isolation of cDNA clones containing *bcr* and *abl* sequences confirms this finding¹². Because the *bcr* part of the chimaeric mRNA could be required to induce the transforming activity of the human *c-abl* oncogene, we have now initiated studies to characterize the normal 'bcr gene' and to determine the effect of a translocation within its coding domain. We demonstrate that as a result of the Ph' translocation, a variable number of *bcr* exons are included in the chimaeric *bcr/abl* mRNA. The *bcr* gene sequences in this mRNA could be responsible for the transition of the *abl* cellular proto-oncogene into an oncogene.

The largest cDNA, V1-3, containing an insert of 2.2 kb, was characterized in detail by restriction enzyme mapping (Fig. 1a) and sequence analysis (Fig. 1b). The cDNA contains one long open reading frame, starting at the poly(G) tail at the 5' end and continuing to nucleotide 1,770, where a stop codon is encountered. All other reading frames have many stop codons within the entire region. The long open reading frame has the coding capacity for 589 amino-acid residues, corresponding to a protein of relative molecular mass (M_r) ~65,000; at the 3' end, a polyadenylation signal occurs at nucleotide 2,182 followed by a poly(A) tail beginning at base 2,208 indicating that the cDNA contains the complete 3' end of the gene. Although translational start sequences are encountered at the 5' end, it is unlikely that this cDNA contains a complete copy of the mRNA, as Northern blot hybridizations indicate the presence of *bcr* mRNAs of ~4.0 and ~6.5 kb. Computer searches of newly isolated protein sequences derived directly from proteins or deduced from cDNA nucleotide sequences frequently result in the identification of proteins with partial homology. Such information is valuable, frequently allowing the assignment of a preliminary function to an unknown protein. Therefore, the PIR FASTP program¹⁰ was used to search for *bcr*-homologous proteins; no proteins with significant homology were found, indicating that the *bcr* protein exhibits an as yet unidentified cellular function.

To determine the orientation of the *bcr* gene on chromosome 22, 5' and 3' probes were prepared from the V1-3 cDNA and hybridized to cosmids⁷ containing human chromosome 22 sequences. This established that the 5' end of the *bcr* gene is towards the centromere of chromosome 22 and is retained after the Ph' translocation; the 3' end of the *bcr* gene lies in the direction of the telomere and is translocated to chromosome 9 in the t(9; 22) translocation. The cDNA hybridizes to restriction enzyme fragments distributed over a region of up to 45 kb of chromosome 22 DNA (Fig. 2a). Within this region, a minimum of 13 exons are present. To determine the exact position and number of exons within the breakpoint cluster region, all hybridizing regions in *bcr* were sequenced and compared with the V1-3 cDNA. Four relatively small exons, designated 1-4, were present within *bcr*, varying in size from 76 to 105 base pairs (bp) (Fig. 2b); in the cDNA, these exons correspond to nucleotides 483-836 (Fig. 1b). As *bcr* was defined as the area on chromosome 22 in which the Ph' breakpoints are found, we conclude that the breakpoints occur within a gene.

Having determined the position of the exons within *bcr*, we investigated whether the breakpoints occur in exon or intron regions. For CML DNA such as that of CML patient C481, this was readily determined. We had previously demonstrated⁷ a breakpoint within a 1.2-kb H/Bg *bcr* fragment in several CML DNA samples, including that of patient C481. As no coding sequences are located within this region (see Fig. 2), patients such as C481 must have a chromosomal breakpoint in the intron between the exons designated 3 and 4.

A less simple situation was encountered in the DNA of patients 0311068 and 7701C. Nonetheless, cloning of 9q⁺ breakpoint fragments from these DNAs (data not shown) and restriction enzyme analysis followed by Southern hybridization enabled us to locate the breakpoints between exons 2 and 3 (see Fig. 2). The breakpoints in the previously cloned^{3,7} 9q⁺ breakpoint fragments of patients 0319129 and 02120185 were analysed by DNA sequencing. In addition, we cloned the 22q⁻ breakpoint

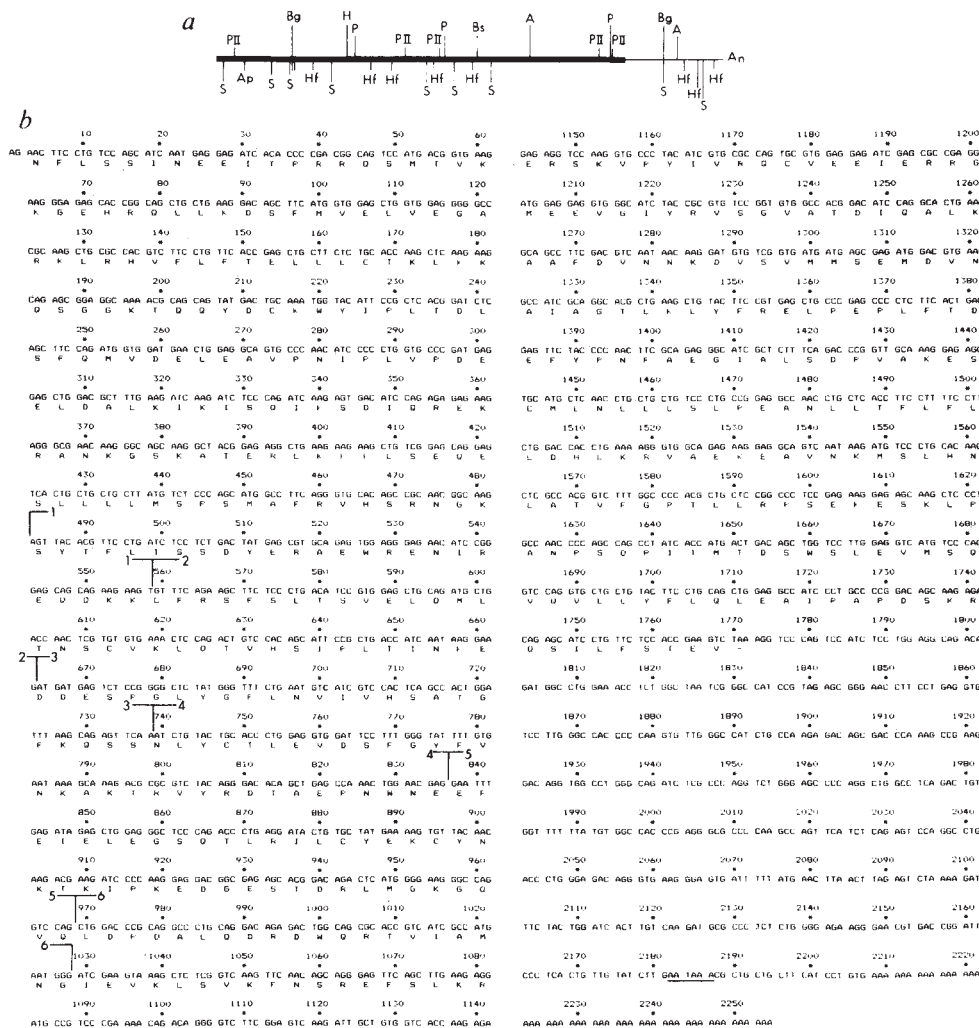


Fig. 1 *bcr* cDNA V1-3. *a*, Restriction enzyme map of V1-3. The black bar indicates the open reading frame sequences, the thin line non-translated sequences. The 3' end of the cDNA containing the poly(A) tail is depicted as A_n. Restriction enzymes include: A, *Ava*I; Ap, *Apa*I; B, *Bgl*II; Bs, *Bst*EII; H, *Hind*III; Hf, *Hin*II; P, *Pst*I; PII, *Pvu*II; S, *Sau*3A. *b*, DNA sequence and translation of V1-3 sequence. Amino-acid residues are indicated with a one-letter symbol below the sequence. The exact position of exons designated 1-6 (see Fig. 2a) within V1-3 *bcr* cDNA is shown by the numbers above the sequence. DNA sequencing was performed using the dideoxy-chain termination method¹⁴ on restriction enzyme fragments of the cDNA subcloned into M13 phage¹⁵. All regions were sequenced on both strands.

fragments of patient 0319129 (unpublished results) and of the cell line K562. DNA sequence analysis of the breakpoint regions of these fragments and that of the sequence of the corresponding chromosome 22 regions enabled us to define the point of translocation for these DNAs (see Fig. 2). None of the breakpoints analysed here could be located within an exon, indicating that in the Ph' translocation, breakpoints occur within intron regions of *bcr*. For four of the six DNA samples analysed, the translocation would result in the transcription of a mRNA that includes *bcr* exons 1 and 2 (see Fig. 2); in two CML DNAs the third exon would be additionally incorporated in the chimaeric *bcr/c-abl* mRNA. Exon 3 encodes only 25 amino acids, not substantially increasing the size of the chimaeric protein.

A knowledge of the DNA sequence of the translocation junction point may provide additional information about the mechanism of chromosomal translocation. Figure 3a shows the sequence of the region containing the crossover point for translocation in the DNA of patient 0319129 and compares this with normal chromosome 22 and 9 DNA sequences. In 0319129 DNA, the chromosomal break has occurred in a rather 'precise' manner, leading to the generation of a 22q⁺ and 9q⁺ sequence exactly reflecting the sequence of the normal chromosome 22 and 9 DNA sequences. However, at the breakpoint, a C nucleotide is found in both the 9q⁺ and 22q⁺ sequences, whereas the chromosome 22 sequence contains a G at that position. Note that both chromosomes 9 and 22 contain limited stretches of homology near the break (Fig. 3a underlined); a DNA search revealed homology of this region to human *Alu* repetitive sequences.

No such similar stretches of homologous sequences between chromosomes 9 and 22 occur at the breakpoint in the DNA of

patient 0212015 (Fig. 3b) or in that of K562 DNA (data not shown). In the 9q⁺ DNA of patient 0212015, sequences are found between the breakpoints on chromosomes 9 and 22 (Fig. 3b) which are not present in the sequenced region of the normal chromosomes 9 and 22. This suggests that in the 9q⁺ chromosome a secondary event has taken place; it is possible that after translocation of chromosome 22 sequences to chromosome 9, a deletion affecting chromosome 9 sequences occurred. The arrow indicating the breakpoint on chromosome 9 (see Fig. 3b) would then represent the point of deletion. In addition, compared with the normal chromosome 9 sequences, the chromosome 9 sequences of the 9q⁺ fragment contain 13 nucleotide changes within an 81-bp stretch. These changes may reflect differences between individuals (the control chromosome 9 sequences were isolated from a human lung carcinoma cosmid library); however, the number of nucleotide substitutions would be very high in such an event. Moreover, the chromosome 22 part of the 9q⁺ fragment contains no nucleotide changes in either intron or exon sequences, favouring the explanation of inefficient DNA repair on deletion of chromosome 9 sequences.

These results indicate that *bcr* is part of a gene oriented with its 5' end towards the centromere of chromosome 22. In the Philadelphia translocation, a break occurs within *bcr*, and sequences from chromosome 9 which contain the human *c-abl* oncogene in all Ph'-positive cases examined^{7,9} are translocated to the 3' of the truncated *bcr* gene. The joining of *bcr* and *c-abl* sequences is highly specific for CML, as this configuration has been found in complex translocations⁹ and even in the leukaemic cells of one CML patient cytogenetically lacking the Ph' chromosome¹¹. Because the orientation of *c-abl* on chromosome 9 is also centromere-5'-3'-telomere, *bcr* and *c-abl* are joined in a

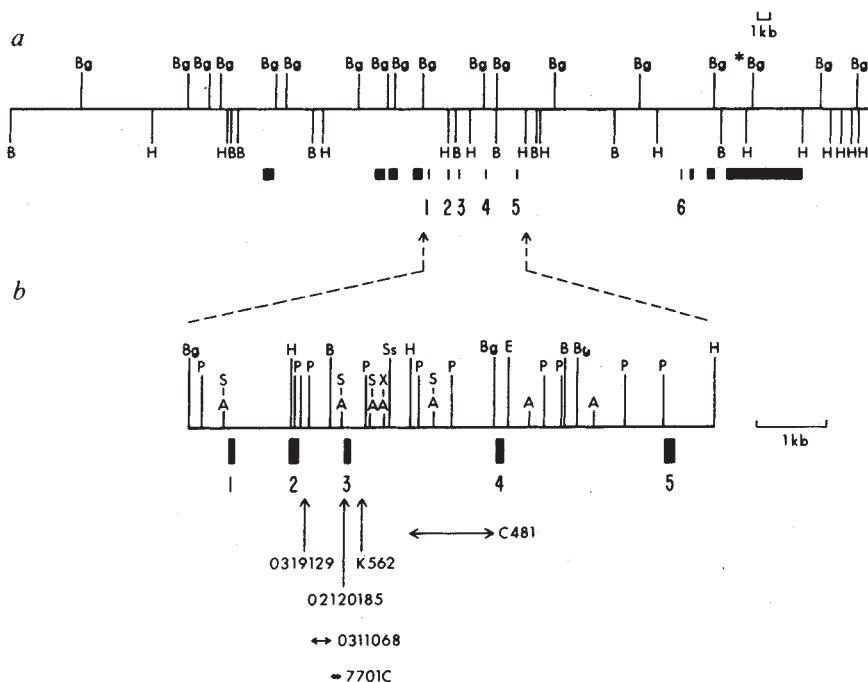


Fig. 2 Genomic organization of the *bcr* gene. *a*, Restriction enzyme map of chromosome 22 sequences encompassing *bcr*. Exons are indicated by black boxes below the restriction enzyme map. The position of the numbered exons has been determined by sequencing; all other exons were located by hybridization to the V1-3 cDNA. The asterisk indicates a polymorphic *Bgl*II restriction enzyme site. *b*, Restriction enzyme map of the breakpoint cluster region, with the exons as indicated in *a*. Below the map, the approximate positions of the breakpoints in different CML DNAs are indicated by horizontal or vertical arrows. Restriction enzymes include: A, *Ava*I; B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hind*III; P, *Pst*I; S, *Sma*I; Ss, *Sst*I; X, *Xho*I. The breakpoint of the 22q⁻ chromosome from DNA 0319129 was cloned as a 9.5-kb *Bgl*II fragment in Charon 30 according to previously described methods¹⁶. 9q⁺ fragments were isolated by cloning a 7.2-kb *Bam*HI fragment and a 7.7-kb *Eco*RI fragment into Charon 30 and λgtw10, respectively.

head-to-tail fashion on the Ph' chromosome. Although the distance between the most 5' *v-abl* homologous to *v-abl* and the physical breakpoint may vary from 14 kb (patient 0319129) to >100 kb (K562), the effect of the translocation on the expression of the *bcr* gene and *c-abl* seems to be very similar in different patients: in K562 and in Ph'-positive CML patients, abnormal RNA transcripts of ~8.5 kb are detected, which hybridize to both *c-abl* and 5' *bcr* exon probes. The molecular cloning of a chimaeric cDNA from K562 cells has provided definitive proof for the existence of chimaeric mRNA¹². The chimaeric mRNAs must be the result of transcription initiating at the promoter of the *bcr* gene; depending on the exact location of the breakpoint, the transcript will include all 5' exons in addition to either exons 1 and 2 or exons 1, 2 and 3 of *bcr*. Recent sequence analysis of K562 *bcr/abl* cDNA confirms the variable presence of the third exon. In K562 we found that, as predicted, the third exon is present in the cDNA, immediately preceding *c-abl* sequences. In contrast, Southern blot analysis of the molecularly cloned 22q⁻ genomic DNA fragment of, for example, patient 0319129 unambiguously demonstrates that in this patient exon 3 has been removed from the Ph' chromosome and will not be included in a chimaeric *bcr/abl* transcript. Transcription continues into

the *c-abl* oncogene, including, as a minimum, the most 5' *v-abl* homologous exon and all exons 3' of it, including the phosphotyrosine acceptor site¹³. We do not know whether the inclusion of exon 3 in the chimaeric mRNA has an effect on the progression of the disease.

Chromosomal aberrations may be generated by specific events involving recombination-prone DNA sequences. Alternatively, such recombination events could occur almost at random. In either case, a very limited number of translocations will result in gene alterations leading to the disruption of normal growth and differentiation. In the Ph' translocation, we have found that breakpoints on chromosome 9 are spread over a region of up to 100 kb. The breakpoints on chromosome 22 occur within a smaller region of around 5.0 kb. Nonetheless, no sequence homology can be found between breakpoint regions of different CML patients or coding regions of *c-abl* and *bcr* genes. Therefore, we may conclude that the processes underlying the Ph' translocation are random recombination events. Once such recombinations result in a genomic configuration that allows the transcription of chimaeric *bcr/abl* mRNA, malignant proliferation of specific cell types may occur. It seems highly likely that this chimaeric mRNA is translated into protein because an

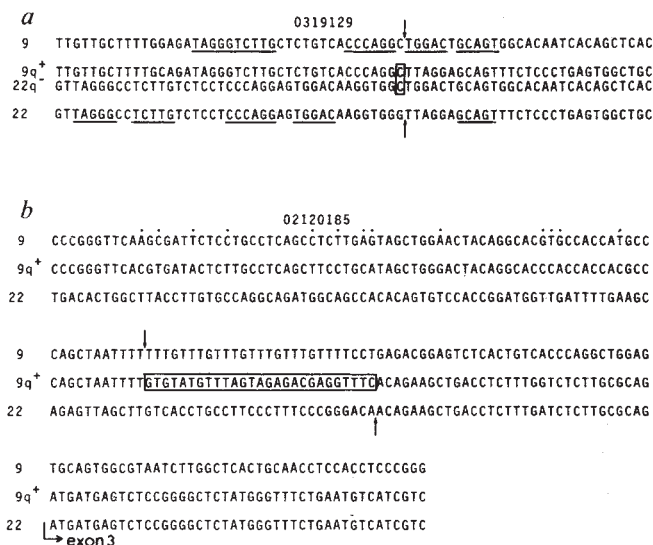


Fig. 3 Breakpoint sequences of the DNAs of two CML patients. *a*, Sequence of 0319129 DNA; the sequences are in a 5'-3' orientation. Normal chromosome 9 sequences (first line) are from non-CML DNA; the 9q⁺ and 22q⁻ sequences (second and third lines) are from DNA of patient 0319129. Normal chromosome 22 sequences (fourth line) are from non-CML DNA. An arrow indicates the breakpoint on chromosomes 9 and 22; the nucleotide C found in both the 9q⁺ and 22q⁻ sequences at the breakpoint is boxed. Limited regions of homology between the normal chromosome 9 and 22 sequences are underlined. *b*, Breakpoint sequence of 02120185 DNA; normal chromosome 9 and 22 sequences (first and third lines in each set) were from non-CML DNA. The 9q⁺ sequence on the second line contains an area boxed to indicate that it does not originate from the normal chromosomes 9 or 22 sequenced in the present experiments. Dots above the chromosome 9 sequences indicate nucleotide differences at those positions from the 9q⁺ chromosome. The beginning of exon 3 (see Fig. 2b) in the 9q⁺ and 22q⁻ sequence is indicated in the figure. Small restriction enzyme fragments containing the breakpoints were chosen for sequence analysis, based on restriction enzyme mapping data and comparison with normal chromosome 9 and 22 maps.

abnormally sized 210,000-*M_r*, *c-abl* protein was detected in K562 cells. In contrast to the normal *c-abl* protein, the P210 has tyrosine kinase activity⁶.

It is tempting to speculate that the *bcrl* moiety of the fusion protein is responsible for this effect. However, although the consequences of the Ph' translocation on a molecular level are becoming evident, it remains to be established whether this phenomenon is actually the cause or merely one of the steps that eventually result in CML.

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Cloning, sequencing and expression of cDNA for a novel subunit of acetylcholine receptor from calf muscle

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The nicotinic acetylcholine receptor (AChR) from fish electric organ has a subunit structure of $\alpha_2\beta\gamma\delta$, and this is thought to be also the case for the mammalian skeletal muscle AChR¹⁻³. By cloning and sequencing the complementary or genomic DNAs, we have previously elucidated the primary structures of all four subunits of the *Torpedo californica* electroplax⁴⁻⁶ and calf muscle AChR⁷⁻¹⁰ and of the α - and γ -subunits of the human muscle AChR^{7,11}; the primary structures of the γ -subunit of the *T. californica* AChR¹² and the α -subunit of the *Torpedo marmorata* AChR^{13,14} have also been deduced elsewhere. We have now cloned DNA complementary to the calf muscle messenger RNA encoding a novel polypeptide (the ϵ -subunit) whose deduced amino-acid sequence has features characteristic of the AChR subunits and which shows higher sequence homology with the γ -subunit than with the other subunits. cDNA expression studies indicate that the calf ϵ -subunit, as well as the calf γ -subunit, can replace the *Torpedo* γ -subunit to form the functional receptor in combination with the *Torpedo* α -, β - and δ -subunits.

The initial cDNA clone for the calf ϵ -subunit was isolated by cross-hybridization with a calf γ -subunit cDNA probe in an experiment aimed at cloning an upstream cDNA sequence for the calf γ -subunit by primer extension (see Fig. 1 legend). Figure 1 shows the nucleotide sequence of the mRNA encoding the calf ϵ -subunit precursor, deduced from the cDNA sequence, together with the predicted primary structure of the polypeptide.

We deduce that the ϵ -subunit precursor consists of 491 amino acids including a hydrophobic prepeptide¹⁵ of 20 amino acids, and calculate the relative molecular masses of the ϵ -subunit and its precursor to be 52,568 and 54,562, respectively.

Figure 2 shows the alignment of the amino-acid sequences of the calf ϵ -subunit precursor and the calf, human and *T. californica* γ -subunit precursors. The mature calf ϵ -subunit shows 53, 53 and 57% sequence homology with the mature calf, human and *Torpedo* γ -subunits, respectively, whereas the degrees of its homology with other mature AChR subunits are as follows: calf, human and *Torpedo* α -subunit, 30% (refs 4, 7), calf β -subunit, 37% (ref. 8), *Torpedo* β -subunit, 39% (ref. 5), calf δ -subunit, 45% (ref. 10) and *Torpedo* δ -subunit, 44% (ref. 5). Homology among the mature γ -subunits is 92%, 56% and 55%, respectively, for the calf/man, calf/*Torpedo* and man/*Torpedo* pairs. Because the calf ϵ -subunit shows similar degrees of homology with the calf, human and *Torpedo* γ -subunits, the divergence of the ϵ - and γ -subunits may have occurred around the time of separation of mammals and fish. Thus, it is possible that fish have a second, undetected gene encoding a γ/ϵ -family subunit, which may or may not now be active.

The calf ϵ -subunit shares structural features common to all four subunits of the fish and mammalian AChR^{4-12,14}. It contains four strongly hydrophobic segments^{4-12,14} (M1-M4) and an amphipathic segment¹⁶⁻¹⁸ (MA). Our expression studies using site-directed mutagenesis of the α -subunit cDNA indicate that these five putative α -helical transmembrane segments are all required for the operation of the ionic channel¹⁸. The hydrophilicity profile¹⁹ and the predicted secondary structures²⁰ of the calf ϵ -subunit are also similar to those of the other AChR subunits^{4-12,21}. These findings suggest that the ϵ -subunit is oriented across the membrane in the same manner as the four other subunits. Three cysteine residues (aligned at positions 128, 142 and 231) and one potential *N*-glycosylation site²² (aligned at position 141) are conserved in all AChR subunits whose sequences are known⁴⁻¹⁴. The calf ϵ -subunit contains two additional potential *N*-glycosylation sites (aligned at positions 66 and 307), one of which (position 66) is assigned to the extracellular side of the membrane^{4-12,14}. Because our expression studies are consistent with the presence of a disulphide bridge between the conserved cysteine residues 128 and 142 of the α -subunit¹⁸, the corresponding cysteine residues of the ϵ -subunit may also form a disulphide bond.

To examine whether the ϵ -subunit can function as an AChR subunit, we synthesized the mRNA specific for this subunit (or the γ -subunit) *in vitro*^{23,24} using the cDNA template and injected the mRNA, combined with the *Torpedo* α -, β - and δ -subunit-specific mRNAs synthesized similarly¹⁸, into *Xenopus* oocytes. The oocytes were then tested for response to acetylcholine (ACh) applied ionophoretically by recording the intracellular potentials. All of the 61 oocytes injected with the calf ϵ -subunit and the three *Torpedo* mRNAs and 38 of the 56 oocytes injected with the calf γ -subunit and the three *Torpedo* mRNAs responded to ACh (Fig. 3) with mean ACh sensitivities (for the responsive oocytes) of 510 and 8 mV μC^{-1} , respectively. None of 60 control oocytes injected only with the *Torpedo* α -, β - and δ -subunit-specific mRNAs was responsive (<0.2 mV μC^{-1}). The mean ACh sensitivity of 70 responsive oocytes out of 72 oocytes injected with the *Torpedo* α -, β -, γ - and δ -subunit-specific mRNAs¹⁸ was 1,300 mV μC^{-1} . The atropine-resistant and (+)tubocurarine-sensitive nature of the observed ACh responses (Fig. 3) indicates the formation of the nicotinic AChR. These results show that the calf ϵ -subunit, as well as the calf γ -subunit, can be functionally substituted for the *Torpedo* γ -subunit.

Blot hybridization analysis using ϵ -subunit cDNA probe of poly(A)⁺ RNA from the skeletal muscle of a 4-month-old fetal calf revealed a hybridization-positive species with an estimated size of ~4,200 nucleotides (Fig. 4A, lane a). No hybridizable species was detected, however, in poly(A)⁺ RNA from the skeletal muscle of a 7-month-old fetal calf (Fig. 4A, lane b), a 9-month-old fetal calf or a newborn calf or from adult bovine diaphragm (data not shown).

[illegible]

both the γ - and ε -subunit mRNAs in fetal calf muscle, like that of the β -subunit mRNA⁸, decreases with increasing age of the calf (Fig. 4), although the relative contents of the ε - and γ -subunit mRNAs in other age groups were not studied systematically. Muscle contains much smaller amounts of ε -subunit mRNA than of γ -subunit mRNA (Fig. 4), and the latter is present in similar amounts to the α -, β - and δ -subunit mRNAs⁷⁻¹⁰. It thus remains uncertain whether the differential presence of the γ - and ε -subunits is responsible for the difference in the junctional and extrajunctional forms of the AChR. Alternatively, the ε -subunit (or the γ -subunit) may be a

Fig. 1 (opposite) Primary structure of the mRNA encoding the ϵ -subunit precursor of the calf muscle AChR. The nucleotide sequence of the mRNA was deduced by sequence analysis²⁸ of the cDNA inserts in clones pcACR ϵ 64, pcACR ϵ 1 and pcACR ϵ 2. Nucleotide residues are numbered in the 5' to 3' direction, beginning with the first residue of the codon specifying the amino-terminal residue of the mature ϵ -subunit, and the nucleotides on the 5' side of residue 1 are indicated by negative numbers. Because the 3'-untranslated sequence presented contains no polyadenylation signal²⁹ and is not followed by a poly(A) tract, this sequence is incomplete, as is the 5'-untranslated sequence shown. The amino-acid sequence, deduced by using the reading frame of the mRNA corresponding to the known amino-acid sequences of the AChR subunit precursors⁴⁻¹⁴, is shown above the nucleotide sequence. Amino-acid residues are numbered beginning with the amino-terminal residue of the mature ϵ -subunit, and the preceding residues are indicated by negative numbers. The possibility that the initiating methionine is located upstream of the 5' end of the cDNA sequence shown cannot be excluded. The nucleotide differences observed between the individual clones are as follows: G (pcACR ϵ 64) or A (pcACR ϵ 2) at position -46; A (pcACR ϵ 64) or C (pcACR ϵ 1) at position 235; the G at position 279 and the C at position 285 of pcACR ϵ 1 (instead of A and T, respectively, in pcACR ϵ 64), derived apparently from the synthetic primer, are not shown. **Methods.** Total RNA was extracted from newborn or fetal calf skeletal muscle, and poly(A)⁺ RNA was isolated by oligo(dT)-cellulose chromatography. The oligodeoxyribonucleotide 5'-GTTGTTCTCCAGCAC-3', complementary to residues 271-285 of the calf γ -subunit mRNA⁹, was synthesized by the triester method and used for specific priming of reverse transcription. Single-stranded cDNA was synthesized using 5 μ g of this primer and 100 μ g of newborn calf muscle poly(A)⁺ RNA as template and was converted to double-stranded cDNA, which was cloned in pBR322 plasmid by inserting it into the *Pst*I site using poly(dG)·poly(dC) homopolymeric extensions. *Escherichia coli* χ 1776 or HB101 was used for transformation, and $\sim 3 \times 10^5$ tetracycline-resistant transformants were screened by hybridization at 60 °C with the *Hinc*II(88)/*Ava*II(202) fragment excised from clone pcACR γ 5 (ref. 9) to yield one positive clone (pcACR ϵ 1 carrying nucleotides 84-285); the restriction endonuclease sites are identified by numbers indicating the 5'-terminal nucleotide generated by cleavage; all hybridization probes were labelled by nick-translation with [α -³²P]dCTP. Another batch of cDNA clones prepared similarly ($\sim 1.5 \times 10^5$ transformants) was then screened by hybridization at 60 °C with the *Mbo*II(152)/*Fnu*4HI(260) fragment from clone pcACR ϵ 1 to give one positive clone (pcACR ϵ 2 carrying nucleotides -46 to 203). The procedures used for the above experiments have been described previously³⁰. A cDNA library was next constructed by the procedure of Gubler and Hoffman³¹, using 10 μ g of oligo(dT)₁₂₋₁₈ primer and 13 μ g of a fraction of fetal (4-month-old) calf muscle poly(A)⁺ RNA ($> 4,000$ nucleotides; see Fig. 4) obtained by centrifugation in a gradient of 5-25% (w/v) sucrose³². Screening of $\sim 8 \times 10^5$ tetracycline-resistant transformants by hybridization at 60 °C with the *Hinf*I(-46)/*Hinf*I(171) fragment from clone pcACR ϵ 2 yielded one positive clone (pcACR ϵ 64 carrying nucleotides -82 to 1,539); the *Hinf*I(-46) site was generated in pcACR ϵ 2 at the junction of the 5' end of the cDNA insert and the poly(dG)·poly(dC) tail.

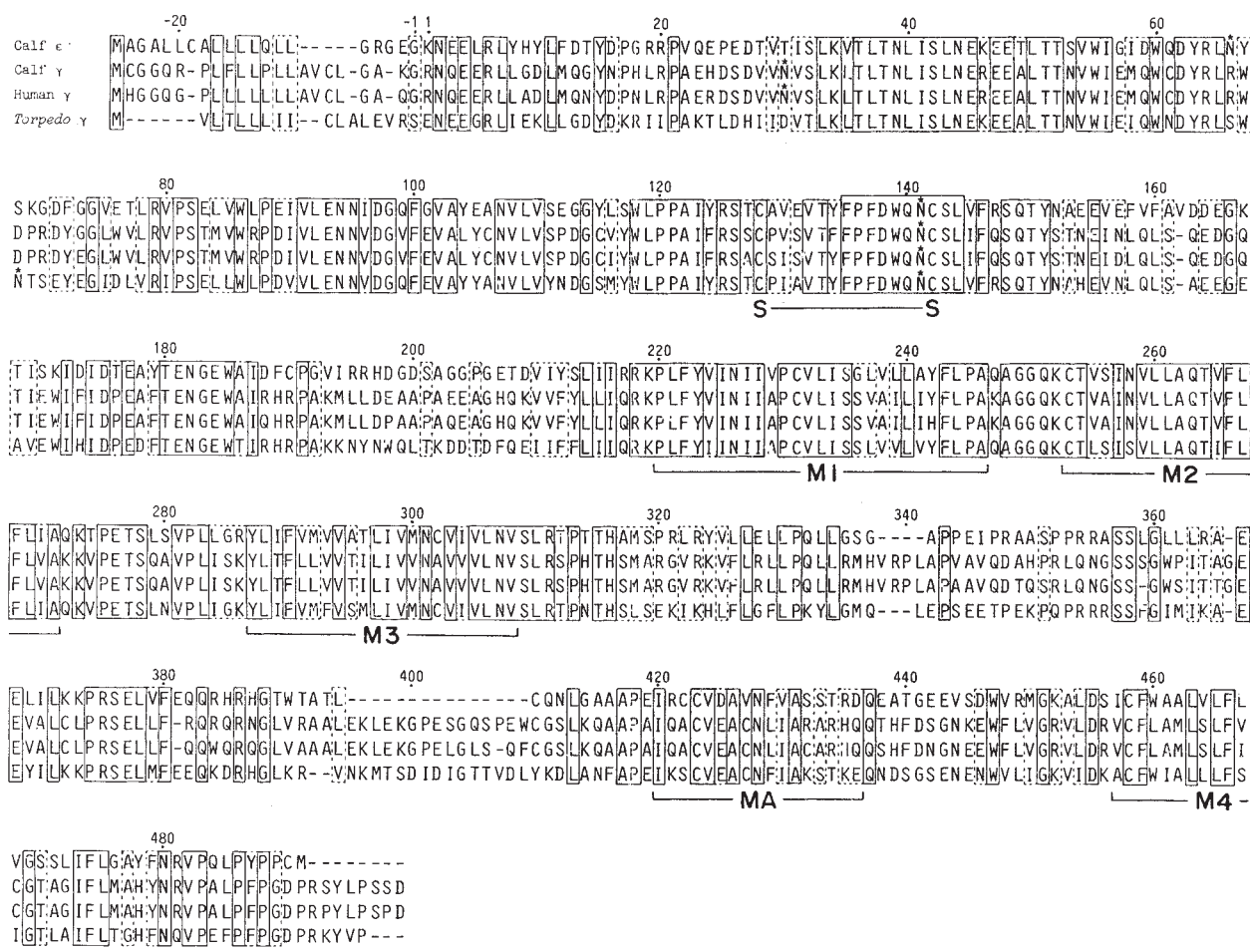


Fig. 2 Alignment of the amino-acid sequences of the calf AChR ϵ -subunit precursor (top) and the calf (second row), human (third row) and *T. californica* AChR γ -subunit precursors (bottom). The one-letter amino-acid notation is used. The sequence data for the calf, human and *T. californica* γ -subunit precursors have been taken from refs 9, 11 and 6, respectively. Sets of four identical residues at one aligned position are enclosed by solid lines, and sets of four identical or conservative residues at one aligned position by dashed lines. Conservative amino-acid substitutions are defined as pairs of residues belonging to one of the following groups: S, T, P, A and G; N, D, E and Q; H, R and K; M, I, L and V; F, Y and W. Gaps (-) have been inserted to achieve maximum homology. The degrees of homology given in the text were calculated on the basis of the alignment of the amino-acid sequences of the 11 AChR subunits (calf, human and *Torpedo* α , calf and *Torpedo* β , calf, human and *Torpedo* γ , calf and *Torpedo* δ and calf ϵ), gaps being counted as one substitution regardless of their length. The positions in the aligned sequences including gaps are numbered beginning with the amino-terminal residue of the mature subunits, and the preceding positions are indicated by negative numbers. The asparagine residues as potential glycosylation sites are marked with asterisks; only those preceding segment M1 are shown. The putative disulphide bridge (S—S) and the putative transmembrane segments (M1, M2, M3, M4 and MA) are indicated; segment MA has been assigned by comparison with that of the *Torpedo* α -subunit defined by deletion mapping¹⁸.

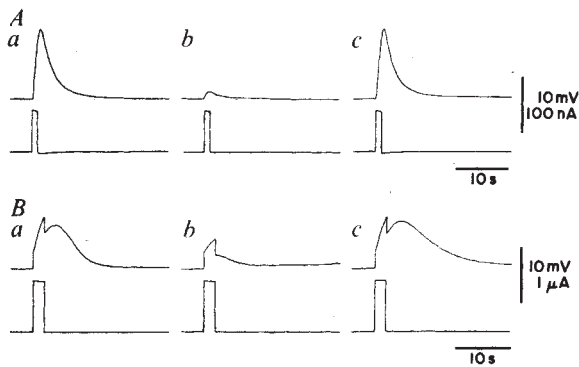


Fig. 3 ACh potentials recorded from *Xenopus* oocytes injected with mRNA specific for the calf AChR ϵ -subunit (A) or γ -subunit (B) together with the *T. californica* AChR α -, β - and δ -subunit-specific mRNAs. The recordings were made at room temperature in Ringer's solution containing 1 μ M atropine and 5 mM $MgCl_2$ instead of $CaCl_2$: upper traces, ACh potentials; lower traces, currents used for iontophoresis of ACh (backing current, 12 nA). The membrane potential was held at -70 mV. Responses to ACh were recorded before (a) and 6–8 min after perfusion with 2 μ M (+)tubocurarine (b) and 8–9 min after washing out the antagonist (c).

Methods. The 320-base-pair (bp) fragment extending from the *Ava*II site generated at the 5' end of the cDNA insert to the *Ava*II(238) site and the *Ava*II(238)/*Eco*RI (1,444) fragment, derived from clone pcACR ϵ 64, and the $\sim$ 520-bp fragment of clone pACR γ 14 (ref. 6) extending from the *Eco*RI(1,474) site to the *Pvu*II site on the vector DNA were ligated. The resulting $\sim$ 2.0-kilobase-pair (kb) fragment was isolated, treated with *E. coli* DNA polymerase I (Klenow fragment) in the presence of dGTP, dCTP and dTTP and inserted into the *Sma*I site of the plasmid pSP64 (ref. 24) in the same orientation as the SP6 promoter to yield the plasmid pSP ϵ c. The *Pst*I(–100)/*Xho*I(207) fragment from clone pcACR γ 11 (ref. 9), the *Xho*I(207)/*Hind*III(1,181) fragment, the *Hind*III(1,181)/*Acc*I(1,560) fragment and the $\sim$ 180-bp fragment extending from the *Acc*I(1,560) site to the *Pvu*II site on the vector DNA, derived from clone pcACR γ 5 (ref. 9), and the $\sim$ 3.0-kb *Sma*I/*Pst*I fragment from pSP64 were ligated. The resulting plasmid pSP γ c, carrying the entire calf γ -subunit cDNA (except for a portion of the 5'-noncoding region) in the same orientation as the SP6 promoter, was identified by colony hybridization with both the above *Xho*I/*Hind*III and *Hind*III/*Acc*I fragments as probes and by restriction endonuclease analysis. The calf AChR ϵ - and γ -subunit-specific mRNAs were synthesized *in vitro*, using *Sac*I-cleaved pSP ϵ c and *Eco*RI-cleaved pSP γ c, respectively, as templates and were then capped *in vitro* with vaccinia virus mRNA guanylyl transferase²³. Each of these mRNAs, combined with the *T. californica* AChR α -, β - and δ -subunit-specific mRNAs¹⁸ (molar ratio of the α -, β -, γ/ϵ - and δ -subunit-specific mRNAs, 2:1:2:1; total mRNA concentration, 120–340 ng μ l^{–1}), was injected into *Xenopus laevis* oocytes (average volume injected per oocyte, $\sim$ 20 nl). The oocytes were incubated for 3 days in conditions described previously³³ before being tested for response to ACh.

component of a third type of AChR which appears at a particular stage of muscle development²⁶. It is also possible that the ϵ -subunit (or the γ -subunit) is a constituent of the nicotinic AChR present in neuronal synapses²⁷.

Conti-Tronconi *et al.*³ have determined the sequences of the amino-terminal 23–35 amino-acid residues of the four polypeptides derived from fetal calf muscle AChR. Three of these sequences agree reasonably well with the amino-terminal sequences of the mature α - (ref. 7), β - (ref. 8) and δ -subunits¹⁰ deduced by us from the cDNA sequences, but the fourth sequence (designated x) matches neither the γ -subunit⁹ nor the ϵ -subunit. This may imply the possible existence of still another subunit of the AChR.

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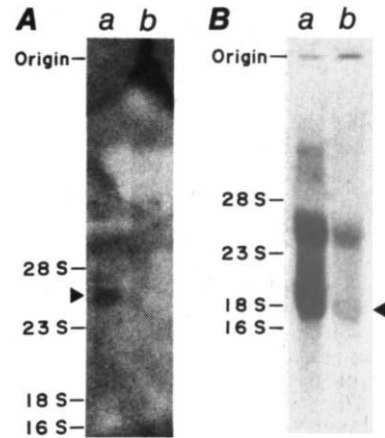


Fig. 4 Autoradiogram of blot hybridization analysis of calf muscle RNA with an AChR ϵ -subunit (A) or γ -subunit (B) cDNA probe. Samples of skeletal muscle poly(A)⁺ RNA (40 μ g each in A or 30 μ g each in B) from a 4-month-old (lane a) or a 7-month-old (lane b) fetal calf were denatured with 1 M glyoxal and 50% dimethyl sulphoxide, electrophoresed on 1.5% agarose gels and transferred to a nitrocellulose filter³⁴ (A) or diazophenylthioether paper³⁵ (B). The hybridization probe used was the 1,535-bp fragment of clone pcACR ϵ 64 extending from the *Pst*I site flanking the 5' end of the cDNA insert to the *Eco*RI(1,444) site (A) or an equimolar mixture of the 1,004-bp fragment extending from the *Pst*I site flanking the 5' end of the cDNA insert to the *Acc*I(1,054) site and the *Acc*I(1,054)/*Acc*I(1,560) fragment, derived from clone pcACR γ 5 (ref. 9) (B); the two probes, labelled by nick-translation with [α -³²P] dCTP, had nearly equal specific radioactivities ($\sim 4 \times 10^8$ c.p.m. per μ g), but the photograph in A is more exposed than that in B, to intensify the hybridization signal. The size markers used were calf and *E. coli* ribosomal RNA. The positions of the ϵ -subunit mRNA ($\sim$ 4,200 nucleotides) (A) and the γ -subunit mRNA ($\sim$ 2,000 nucleotides⁹) (B) are indicated by arrowheads; the larger RNA species ($\sim$ 3,700 nucleotides) hybridizable with the γ -subunit cDNA probe probably represents incompletely spliced RNA as described previously⁹.

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Expression of genes of the T-cell antigen receptor complex in precursor thymocytes

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The antigen receptor on T lymphocytes has recently been characterized as a heterodimeric, transmembrane glycoprotein consisting of disulphide-linked α (acidic) and β (basic) subunits of relative molecular mass (M_r) 40,000–45,000 each. The genes encoding these proteins have been cloned^{1–4} and shown to resemble immunoglobulin genes in both overall structure and the requirement for DNA rearrangement before expression^{5–11}. In humans, three additional proteins, termed the T3 complex, are found associated with the clonotypic receptor, and a role for T3 in receptor expression has been proposed^{12–15}. Despite these recent advances in characterizing the antigen receptor complex, there is as yet little understanding of T-cell maturation, particularly the stage of T-cell ontogeny at which the genes encoding the antigen receptor and its associated structures are expressed and assembled. In the adult, stem cells destined to differentiate into T cells arise in the bone marrow and migrate to the thymus¹⁶, where T-cell precursors proliferate, develop a preference for recognizing antigens in the context of self MHC molecules^{17,18} and are released to the periphery. Recently, cells that have the properties of immature murine thymocytes have been isolated and described^{19,20}. We have now analysed these cells with a series of molecular probes and we describe three distinct patterns of T-cell antigen receptor gene rearrangements in developing thymocytes.

Two-colour flow cytometric analysis of the Lyt2 and L3T4 T-cell-surface antigens on adult murine thymocytes and analysis of T8 and T4 markers on human thymocytes using monoclonal antibody plus complement-mediated lysis reveal four major subpopulations^{19–22} (Fig. 1). In the mouse, cells bearing both Lyt2 and L3T4 make up most thymocytes (80–85%) and correspond to cells found predominantly in the cortex. Immunocompetent, mature thymocytes have one of two phenotypes, Lyt2⁺, L3T4⁺ or Lyt2⁺, L3T4⁺, which make up 3–5% and 8–15% of murine thymocytes, respectively^{20,23}. The fourth subpopulation is negative for both Lyt2 and L3T4 markers and represents only 3–4% of thymocytes. Some of these cells bear low levels of the Ly1 surface antigen, a fact important for their initial isolation²⁰. Thus their phenotype is Lyt2⁺, L3T4⁺, Ly1^{dull} and they have been called dull Ly1 (dLy1) cells. They are large, blast-like cells and may correspond to the blast cells with a similar phenotype that are found early in the developing fetal thymus²³. dLy1 cells isolated with a purity of 97% have several properties indicating that they are T-cell precursors^{19,20}. When cultured *in vitro*, 25–30% differentiate within 20 h to cells with the Lyt2⁺, L3T4⁺ phenotype. This experiment extends the observation of Ceredig *et al.* showing that Lyt2⁺ precursors differentiate *in vitro* to Lyt2⁺ thymocytes²⁴. More importantly, adoptive transfer experiments indicate that these isolated cells re-enter and transiently repopulate the thymus of congenic irradiated recipient mice. Donor-derived cells with both the Lyt2⁺, L3T4⁺ and cortical (Lyt2⁺, L3T4⁺) phenotypes appear first in the thymus. Within 10–20 days after transfer, donor-derived cells have differentiated into thymocytes with the phenotypes of immunocompetent T cells (Lyt2⁺, L3T4⁺ and Lyt2⁺, L3T4⁺). However, cells in the dLy1 population have certain properties which distinguish them from

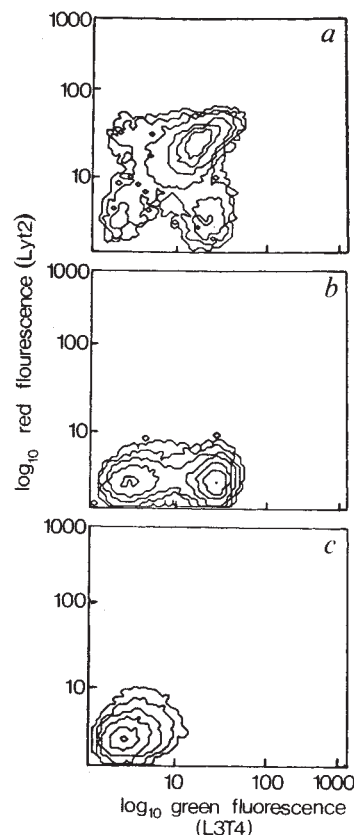


Fig. 1 Two-colour flow cytometric analysis of Lyt2 and L3T4 on B6 thymocytes during the isolation of the precursor thymocyte population. Thymocyte suspensions from C57BL/6 mice (6–8 weeks old) were prepared as described elsewhere²⁰. Cells were incubated for 30 min at 4°C with a saturating concentration of antibody, then washed twice in Hank's balanced salt solution with 0.1% bovine albumin and 0.1% sodium azide and resuspended for the next incubation. The sequence of reagent incubation for panel a was: (1) anti-L3T4 culture supernatant²⁵, (2) fluorescein isothiocyanate-labelled goat anti-rat immunoglobulin, (3) normal rat serum, (4) biotin-labelled rat anti-Lyt2²⁶, (5) Texas-red avidin. Flow cytometry was performed on a fluorescence activated cell sorter¹⁹. a, Unfractionated thymocytes. b, Lyt2⁺ thymocytes, isolated as previously described¹⁹. Briefly, $20\text{--}30 \times 10^8$ thymocytes were incubated with anti-Lyt2.2 ascites fluid and rabbit complement. Viable cells were obtained by purification on Ficoll-Paque (Pharmacia). Cells were washed three times. c, Isolated Lyt2⁺, L3T4⁺, Ly1^{dull} (dLy1) cells. Lyt2⁺ cells were treated with anti-Ly1.2 ascites, purified anti-L3T4 antibody and complement, and again purified by centrifugation over Ficoll.

bone marrow-derived stem cells that can also repopulate the thymus, including a limited capacity for self-renewal, and the ability to give only a single wave of differentiation that generates the other three phenotypes in the thymus. Although studies on the functional properties of these dLy1 cells and their progeny remain to be performed, these data suggest that the dLy1 cells represent a population of precursor thymocytes at an intermediate developmental stage between marrow-derived stem cells and immunocompetent T cells.

The expression of RNA encoding T-cell antigen receptor proteins was assayed in the dLy1 precursor population to determine whether these early thymocytes were transcribing receptor genes. Total cytoplasmic RNA was analysed by Northern blotting with α -(TT11)² and β -(86T5) chain²⁶ cDNA probes (Fig. 2). These experiments reveal that the dLy1 cells as well as unfractionated thymocytes contain the 1.3- and 1.0-kilobase (kb) β -gene transcripts shown to represent V-D-J and D-J rearrangements respectively^{9,10}. In this experiment the precursor (dLy1) cells contained β -chain-specific transcripts in a concentration approximately equal to the unfractionated cell. This indicates that within the highly purified dLy1 population, at

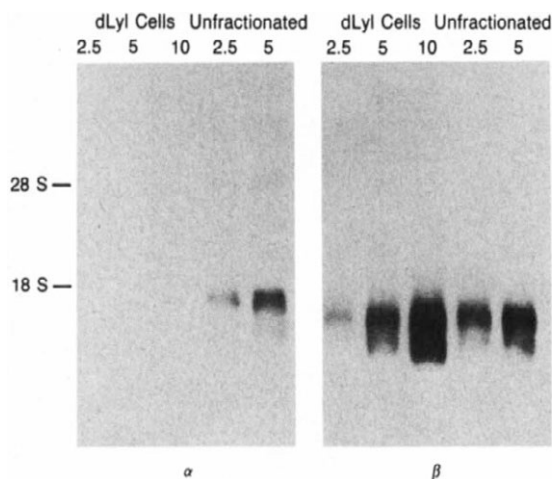


Fig. 2 Northern blot analysis of thymocyte and dLy1 RNA to reveal α - and β -chain gene expression. Cells ($0.9\text{--}2.5 \times 10^8$) were homogenized in 4 M guanidinium thiocyanate and RNA was purified by centrifugation through CsCl^{29} . The RNA was denatured in 2.2 M formaldehyde, and the indicated amount of RNA (μg) electrophoresed in a 1% agarose gel with 0.2 M formaldehyde, then transferred to nitrocellulose. The probe used to detect α -chain gene expression was the *Rsa*I to *Avu*II fragment comprising the constant region of the TT11 cDNA clone². The 86T5 cDNA clone was used to detect β -chain gene expression²⁷. Probes were labelled by nick-translation. Hybridization was in $5 \times \text{SSPE}$, $1 \times \text{Denhardt's}$ solution, 10% dextran sulphate and 50% formamide at 42°C . The blots were washed twice in $2 \times \text{SSPE}$, 0.1% SDS at 55°C and once in $0.2 \times \text{SSPE}$ at 55°C .

least some cells transcribe RNA for the β -chain gene product. When the same blot was probed with the α -chain cDNA clone TT11, however, essentially no hybridizing RNA was observed in the dLy1 population. Since the dLy1 cells is an early thymocyte, these data suggest that α -chain RNA expression follows β -chain gene expression in intrathymic T-cell maturation.

The T-cell antigen receptor in mature T cells is associated with three molecules comprising the T3 complex. A cDNA insert of the clone PEM-T3 δ , which encodes the murine T3 M_r 20,000 glycoprotein δ -chain²⁸, was used to probe the dLy1 thymocyte population (Fig. 3). The probe hybridized to RNA from the purified dLy1 population as well as to RNA from unfractionated thymus and the mature T-cell line CTLL, but not with A20 B-cell RNA. As RNA for this product is expressed in the dLy1 population, this gene product is not likely to be limiting in the assembly of the T-cell antigen/receptor complex. Expression of RNA for the β -chain of the antigen receptor and the T3 δ -chain are thus both early events in T-cell maturation.

To analyse antigen receptor genes of early T cells at a clonal level, we fused purified precursor thymocytes with the drug-marked thymoma BW5147, reasoning that these large, proliferating immature cells would fuse readily with the thymoma line. They would thus behave like pre-B cells derived from fetal liver, which fuse efficiently to plasma cell lines³⁰. Indeed, we observed a high frequency of fusions in such an experiment (1 in $5\text{--}10 \times 10^4$). Twelve of these lines were tested for the presence of the Thy 1.2 allele, a T-cell marker donated by the B6 dLy1 cells and not by the AKR-derived Thy 1.1 BW5147 cells. Of these 12, 9 expressed the expected marker from the input cells, implying, though not proving, that they derived from fusions involving cells of the T lineage³¹. These cells were also stained with antibodies binding Lyt2 and L3T4 and were all negative for these markers, consistent with a dLy1 (Lyt2⁻, L3T4⁻) origin.

DNA was isolated from these 12 lines and subjected to Southern blot analysis (Fig. 4a, b). Germline B6 mouse DNA digested with the restriction enzyme *Pvu*II gave three tightly migrating, though not always well-resolved, bands of 6.4, 6.2 and 6.0 kb hybridizing with the 86T5 β -chain probe. DNA from the BW5147 thymoma cell showed two non-germline bands at 6.5 and 5.8 kb, resulting from two separate rearrangements to

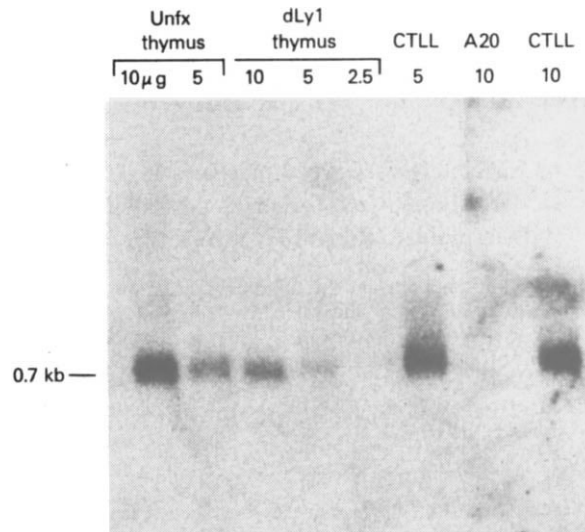


Fig. 3 Northern blot analysis with the T3 δ -chain probe. The RNA and blots were prepared as described in Fig. 2. The blots were prehybridized at 42°C for 2 h in 50% deionized formamide, $5 \times \text{SSC}$, $5 \times \text{Denhardt's}$ solution, 50 mM sodium phosphate, pH 6.5, 0.1% SDS with 250 mg ml^{-1} denatured salmon-sperm DNA. Hybridization was carried out in the same buffer for 16 h at 42°C . The mouse T3 δ -chain cDNA insert of clone PEM-T3 δ ²⁸ was nick-translated and used as a hybridization probe. Following hybridization, blots were washed three times in $2 \times \text{SSC}$, 0.1% SDS at 25°C and twice in $0.1 \times \text{SSC}$, 0.1% SDS at 50°C .

the $C_{\beta}2$ locus (R. Lechler and R.N.G., N. E. Lee and M.M.D., unpublished results). The hybrids have a combination of BW5147-derived bands, germline bands, and additional bands presumably representing rearrangements in the precursor population. Eight hybrids possess such additional rearrangements in their DNA, whereas four Thy 1.2⁺ hybrids (1G8, 1G10, 2C9 and 2E5) contain non-BW5147 hybridizing bands of only germline pattern. To confirm the finding that these four hybrids had only germline bands of B6 origin, further analysis of these DNA preparations after digestion with the restriction enzymes *Hpa*I (Fig. 4b) and *Hind*III (data not shown) was performed. The additional experiments indicated that these four hybrids had all the expected germline and BW5147-derived restriction fragments without any additional bands. Thus an analysis with three restriction enzymes revealed that, while many hybridomas showed β -locus rearrangements consistent with the observed transcription of β -chain genes in the dLy1 population, a substantial fraction of the hybrids retained nonrearranged β -chain genes.

The pattern of α - and β -chain gene expression in the dLy1 and total thymocyte populations, together with these data on β -locus rearrangements in dLy1-derived T hybridomas, suggest three distinct patterns of T-cell antigen receptor gene rearrangement in developing thymocytes. Two of these subpopulations exist within the dLy1 precursor population. The first, characterized on the basis of four hybrids, has germline β -chain and, because α transcripts are absent in dLy1 cells, apparently unrearranged α -chain gene loci. The analysis of genomic DNA from eight additional hybrids defines a second subpopulation with rearranged β -chain, and by the same reasoning, germline α -chain genes. One can infer the existence of a third subpopulation, not present among dLy1 cells, in which both gene loci are rearranged, as thymocytes bearing a mature α/β heterodimer³³ and an intact T3 complex³⁴ have been demonstrated with monoclonal antibodies. Although the T3 δ -chain gene is expressed in the dLy1 subpopulation, no information yet exists to place expression of the other T3 components in this scheme.

The characterization of the first two of these subpopulations depends on certain assumptions about the fusion of dLy1 cells

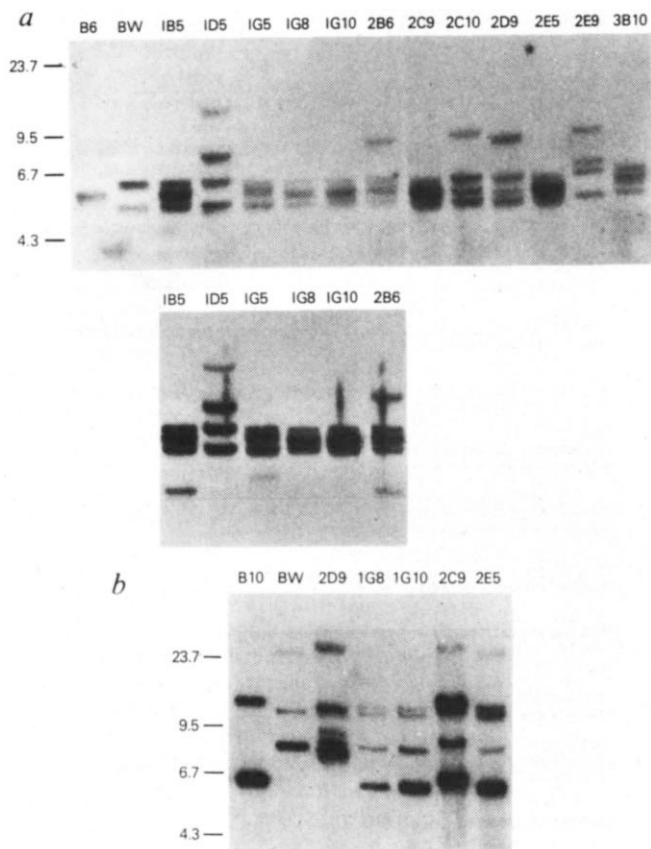


Fig. 4 Southern blot analysis of genomic DNA from dLy1 hybridomas. dLy1 cells were isolated as described in Fig. 1 and fused to the drug marked thymoma BW5147 with polyethylene glycol. Hybridomas were selected in hypoxanthine/aminopterin/thymidine medium. DNA was prepared from the cells by standard methods³², digested with the restriction enzyme *Pvu*II (a) or *Hpa*I (b), electrophoresed through 0.7% agarose and blotted onto nitrocellulose. The 86T5 β -chain probe was used for hybridization and the blots were washed three times in $2 \times \text{SSC}$, 0.1% SDS at 25°C and three times in $0.2 \times \text{SSC}$, 0.1% SDS at 60°C . The inset represents a longer exposure of the blot in panel a. Germline DNA from liver is included in the first lane.

with the BW5147 thymoma line. First, that contaminating cells of other (non-dLy1) phenotypes did not fuse to the BW5147 thymoma. An analysis of those non-dLy1 cells showed that most had a mature phenotype and thus were likely to contain predominantly small resting cells that might not fuse without stimulation (Yeh *et al.*³⁵ and L.E.S., unpublished data). Furthermore, the 12 hybrids randomly chosen for DNA analysis bore the expected $\text{Lyt}2^-$, L3T4^- phenotype of the dLy1 cell. Second, because the dLy1 population seems heterogeneous for a series of other cell-surface markers (B.J.F., unpublished data), it is possible that there was preferential fusion involving a subpopulation of dLy1 cells such as those with germline β -chain loci. The proportion of hybridomas that have germline β -chain genes may not accurately reflect the true fraction of dLy1 cells with this phenotype. We assume, however, that these cells are in the T-cell lineage because all bear the Thy 1.2 surface marker.

Because the relationships among the various thymocyte subpopulations remain disputed¹⁶, it is not possible to make direct correlations between the various patterns of α - and β -chain gene expression described here, and a single, linear pathway of T-cell development. Nevertheless, the three distinct T-cell rearrangement patterns can be related to stages of the better-defined B-cell differentiation process³⁶. In the B lineage, the heavy-chain genes first undergo rearrangement, followed by the appearance of intracellular μ proteins in the pre-B cell. Only after subsequent light-chain gene rearrangement and expression has occurred does the complete immunoglobulin molecule appear on the

surface of the mature B cell. In the T lineage, at some point after commitment, cells with non-rearranged α - and β -chain genes can be found. The four hybrids with germline β -chain gene loci may be examples of such cells, although it remains to be shown that these cells have germline α -chain genes and are not cells of a lineage that do not use the β -chain gene locus (for example, suppressor T cells³⁷). By analogy to B-cell development³⁸, we propose the name 'pro-T' cells for this committed precursor subpopulation. Cells of the second subpopulation are analogous to the pre-B cell. These pre-T cells have rearranged one of their receptor genes, β , and express RNAs corresponding to both *D-J* (1.0 kb) and *V-D-J* (1.3 kb) rearrangements. Although we have no evidence for *D-J* rearrangement preceding *V-D-J* as the 1.0- and 1.3-kb mRNAs are present in equal amounts, such a progression may occur³⁹ as in B cells^{40,41}. At some later stage, the α -chain genes are rearranged and transcribed, giving rise to the complete receptor on the mature T cell. Such a sequence of rearrangements also seems to occur in the fetal thymus⁴². In the experiments of Raulat *et al.*, however, a preponderance of 1.0-kb β -chain RNA is seen in the fetal thymus, perhaps because during ontogeny the developmental steps are better synchronized. The complexity of events within this maturational scheme may account for the large number of β gene clones corresponding to *D-J* type rearrangements that were isolated from a thymocyte library⁹. A *V*-containing β -chain gene product may be needed before α -chain gene rearrangement, accounting for the relatively low level of α -chain transcripts in the thymus². Although cells with characteristics of pro-T and pre-T stages can be found in the immature dLy1 population of thymocytes, and the thymus contains some T cells with mature phenotype and antigen receptors, further experiments are necessary to determine if differentiation through these stages takes place entirely or solely within the thymus. Repopulation experiments with isolated pro-T and pre-T cells should provide further insight into these issues.

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10      20      30      40      50
GCCGCGATC CAGCCGCCAG ACAGACACCA GAGAACCCAC C ATG CCC CCC TTT GAG CCC
MET Ala Pro Phe Glu Pro

71      86      101
CTC GCT TCT GGC ATC CTG TTG CTG TGG CTG ATA CCC ACC AGG GGC TGC
Leu Ala Ser Gly Ile Leu Leu Leu Trp Leu Ile Ala Pro Ser Arg Ala Cys

116     131     146     161
ACC TGT GTC CCA CCC CAC CCA CAG AGG GGC TTC TCC AAT TCC GAC CTC GTC ATC
Thr Cys Val Pro Pro His Pro Gln Thr Ala Phe Cys Asn Ser Asp Leu Val Ile

176     191     206     221
AGG GGC AAG TTC CTG GGG ACA CCA GAA GTC AAC CAG ACC ACC TTA TAC CAG CGT
Arg Ala Lys Phe Val Gly Thr Pro Glu Val Asn Gln Thr Thr Leu Tyr Gln Arg

236     251     266
TAT GAG ATC AAG ATG ACC AAG ATG TAT AAA GGG TTC CAA GGC TTA GGC GAT CGC
Tyr Glu Ile Lys MET Thr Lys MET Tyr Lys Gly Phe Gln Ala Leu Gly Asp Ala

281     296     311     326
GCT GAC ATC CCG TTC CTC TAC ACC CCC GGC ATG CAG AGT GTC TGC GCA TAC TTC
Ala Asp Ile Arg Phe Val Tyr Thr Pro Ala MET Glu Ser Val Cys Gly Tyr Phe

341     356     371
CAC AGG TCC CAC AAC CCG AGC GAG GAG TTT CTC ATT GCT GGA AAA CTG CAG GAT
His Arg Ser His Asn Arg Ser Glu Glu Phe Leu Ile Ala Gly Lys Leu Gln Asp

386     401     416     431
GGA CTC TTG CAC ATC ACT ACC TGC AGT TTT GTG GCT CCC TGG AAG ACC CTC AGC
Gly Leu Leu His Ile Thr Thr Thr Cys Ser Phe Val Ala Pro Trp Asn Ser Leu Ser

446     461     476     491
TTA GCT CAG CCG GGC GGC TTC ACC AAG ACC TAC ACT GTT GCT GAG CAA TGC
Leu Ala Gln Arg Arg Gly Phe Thr Lys Thr Tyr Thr Val Gly Cys Glu Cys

506     521     536
ACA GTG TTT CCC TGT TTA TCG ATC CCG TCC AAA CTG CAG AGT GGC ACT CAT TGC
Thr Val Phe Pro Cys Leu Ser Ile Pro Cys Lys Leu Gln Ser Gly Thr His Cys

551     566     581     596
TTG TGG ACG CAC CAC CTC CTC CAA GGC TCT GAA AAG GGC TTC CAG TCC CCG CAC
Leu Trp Thr Asp Gln Leu Leu Gln Gly Ser Glu Lys Gly Phe Gln Ser Arg His

611     626     641
CTT GCC TGC CTG CCT CCG CAG CCA GCG CTG TGG ACC TGG CAG TCC CTG CCG TCC
Leu Ala Cys Leu Pro Arg Glu Pro Gly Leu Cys Thr Trp Gln Ser Leu Arg Ser

656     675     685     695     705     715
CAG ATA GGC TGA ATCCTGCCCG GAGTGAAGC TGAACCTCC ACAGTGTCCA CCCTGTGCC
Gln Ile Ala

725     735     745     755     765
ACTCCCATCT TTCTTCGGA CAATGAATA AAGAGTTACA CCAGCAAAAA AAAAA

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Molecular characterization and expression of the gene encoding human erythroid-potentiating activity

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Erythropoietin is the primary physiological regulator of erythropoiesis; however, *in vitro* studies have identified another class of mediators which appear to be important in stimulating erythroid progenitors. These factors have generally been referred to as burst-promoting activities (BPA), because they stimulate the growth of early erythroid progenitors referred to as burst-forming units-erythroid (BFU-E) which give rise to colonies of up to thousands of haemoglobinized cells^{1,2}. We recently reported purification of a burst-promoting activity from medium conditioned by the Mo T-lymphoblast cell line infected with human T-cell lymphotropic virus type II (HTLV-II)^{3,4}. This purified glycoprotein of relative molecular mass (M_r) 28,000 also stimulates colony formation by more mature erythroid precursors (CFU-E) and is therefore referred to as erythroid-potentiating activity (EPA)⁵. Purified EPA specifically stimulates human and murine cells of the erythroid lineage, unlike murine interleukin-3 (IL-3) which stimulates precursor cells from all haematopoietic lineages⁶. We report here the isolation of a complementary DNA molecular clone encoding EPA and its use in producing EPA in COS (monkey) cells and CHO (Chinese hamster ovary) cells. We also define the organization of the EPA gene in human DNA.

Purified EPA obtained from two 10-l preparations of medium conditioned by the Mo T-lymphoblast cell line was analysed in four separate samples using a gas phase sequencer. From a total of approximately 25 µg of purified protein, the following partial amino-acid sequence was obtained from the NH₂-terminus:

1 5 10 15 20 25
(A) T C V P P H ? Q T A F C N S D L V I R A K F V G T

Using this sequence, three different oligonucleotide probes were prepared. The first probe consisted of all 64 possible 12-mers encoding residues 9-12 above (QTAF). The second probe consisted of 64 14-mers predicted from residues 21-25 (AKFVG). The third probe was a pool of 16 53-mers predicted from residues 9-26 based on preferred codon usage. A cDNA library was prepared from Mo messenger RNA and enriched for sequences specific to mature T cells by hybridizing the single-stranded

Fig. 1 Nucleotide and deduced amino-acid sequence of the cDNA clone encoding EPA.

Methods. Standard gentle lysis procedures were used to prepare RNA from 2×10^9 lectin-stimulated Mo cells. Polyadenylated RNA was selected with oligo(dT)-cellulose and used as the template to synthesize the first strand of cDNA by reverse transcription. Approximately 1 µg of single-stranded cDNA, obtained from 10 µg of poly(A)⁺ RNA, was enriched by hybridization to 10-fold excess concentrations of mRNA from a human B-cell line (Daudi) and immature T-cell line (CEM), and the unhybridized cDNA (~5% of input cDNA) was recovered by chromatography on hydroxylapatite. Standard techniques were used to synthesize the second cDNA strand, digest the single-stranded loop and add homopolymeric (dC) tails. The cDNAs were annealed with G-tailed pBR322 and used to transform *Escherichia coli*. A final yield of 40,000 colonies was obtained from double-stranded cDNA. EPA was purified from serum-free Mo-conditioned medium using the previously published method³. Two preparations of EPA containing ~25 µg were subjected to Edman degradation and analysed on a gas phase sequencer. Based on the amino-acid sequence obtained, three pools of oligonucleotide probes were constructed. The pools were used to probe replicate filters containing the recombinant plasmids described above. Potential EPA clones were identified by colony hybridization. DNA from positive clones was analysed by restriction digestion and hybridization to the oligonucleotide probes. EPA cDNA clones were verified by DNA sequence analysis using the dideoxynucleotide chain termination procedure primed with the oligonucleotides described in the text¹⁹.

cDNA to mRNA from B cells (Daudi) and immature T cells (CEM). One set of replicate filters containing the enriched Mo cDNA library was hybridized with the 64 12-mers and the other set with the 64 14-mers described above. DNA from 10 clones which hybridized with both probes was prepared and analysed by digestion with *Pst*I and hybridization to each of the three oligonucleotide probes. Three clones, each of which had at least one internal *Pst*I site, hybridized to all three probes, and were further characterized.

A pool of eight strongly hybridizing 14-mers (corresponding to amino-acid residues 21-25) was used as a primer for sequencing the three putative EPA cDNA clones. The resulting DNA sequence was that expected from the amino-acid sequence of

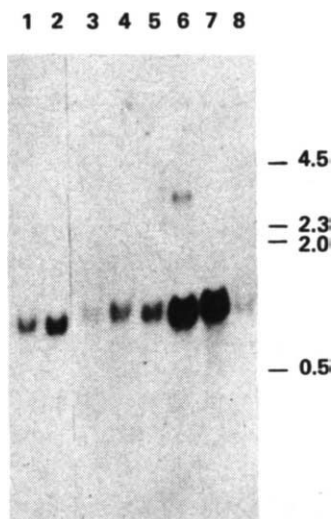


Fig. 2 Analysis of EPA mRNA from human cells and cell lines. Polyadenylated cytoplasmic RNA (5 μ g) was isolated from U-937 cells cultured in the presence of 10 nM dexamethasone for 20 h (lane 1); U-937 cells (lane 2); Mo cells cultured in the presence of dexamethasone (lane 3); Mo cells (lane 4); Mo cells treated for 20 h with phytohaemagglutinin (0.3%) and phorbol myristate acetate (5 mg ml⁻¹) (lane 5); C10-MJ2 cells (lane 6); UCD-144-MLA cells (lane 7); and peripheral blood lymphocytes (lane 8). The mRNA was fractionated on a 1% agarose gel, transferred to nitrocellulose and hybridized with the EPA clone labelled with ³²P as described previously¹².

residues 1-19, confirming that these cDNA clones encode the EPA protein.

The complete nucleotide sequence of all three clones was obtained by dideoxy sequencing of M13 subclones (Fig. 1). The cDNA encodes a protein of 207 amino acids. Based on the NH₂-terminal sequence of the mature protein, a signal peptide of 23 amino acids is predicted by the nucleotide sequence. Characteristic of other signal peptides, this region is rich in hydrophobic amino acids. The predicted relative molecular mass of the mature protein is in good agreement with the apparent 18,000 *M_r* observed following digestion of EPA with endoglycosidase F (ref. 4). Endoglycosidase F removes both high mannose and complex asparagine-linked carbohydrate moieties⁷; two possible sites for asparagine-linked glycosylation are amino acid 53 and 101. The presence of 12 cysteine residues in the deduced amino-acid sequence predicts a protein structure which could account for the marked heat stability previously described for EPA biological activity⁵.

The EPA cDNA clone was used to screen a cDNA library prepared from the gibbon lymphosarcoma cell line UCD-144-MLA⁸, a source of numerous lymphokines including burst-promoting activity. The resulting clones were sequenced and compared with the nucleotide sequence of human EPA. Seven nucleotides within the coding region differed between human and gibbon EPA, resulting in a single amino acid change from arginine to lysine at residue 43.

Further confirmation that these clones encode EPA was obtained by Northern analysis. mRNA was prepared from cells previously shown to produce burst-promoting activity. Figure 2 shows that Mo (lane 4), C10-MJ2⁹ (lane 6) and UCD-144-MLA⁸ (lane 7) mature T-lymphoblast cell lines, peripheral blood lymphocytes (lane 8) and the U-937¹⁰ (lane 2) monocytic cell line produce a 0.9-kilobase (kb) transcript which hybridizes with the EPA cDNA probe. Erythroid-potentiating activity mRNA could not be detected in samples from the immature B-lymphoblastoid cell line Daudi (data not shown). These results are in agreement with previous reports of EPA activity, as well as the presence or absence of the EPA protein in cell supernatants as determined by immunoprecipitation and radioimmunoassay

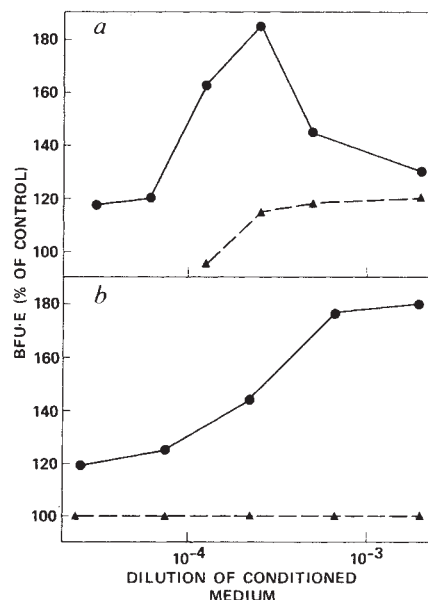


Fig. 3 Biological activity of EPA cDNA clones expressed in mammalian cells. *a*, Stimulation of human peripheral blood BFU-E by dilutions of supernatants containing recombinant EPA expressed transiently in COS cells (●) compared with mock-transfected COS cells (▲). *b*, Supernatant from CHO cells which constitutively produce high levels of recombinant EPA (●) compared with supernatant from a CHO line which does not express the EPA cDNA (▲).

Methods. The expression vector 91023(B) is described in detail elsewhere¹⁷. This plasmid contains eukaryotic regulatory elements from adenovirus, simian virus 40, and the mouse immunoglobulin and dihydrofolate reductase genes. COS 7 monkey cells were transfected with the EPA cDNA inserted into the 91023(B) vector using a previously described procedure¹². Briefly, cells were incubated for 12 h at 37 °C in serum-free Dulbecco's modified Eagle's medium containing DEAE-dextran (*M_r* 5 × 10⁶ Pharmacia, 250 mg ml⁻¹) with 0.1 M Tris pH 7.3 and 2 μ g ml⁻¹ plasmid DNA. After the incubation, cells were rinsed with serum-free medium and treated for 2 h at 37 °C with 1 mM chloroquine in medium containing serum. Cells were washed and medium was collected at varying intervals. Chinese hamster ovary cells (CHO) were transfected with the 91023(B) vector containing the EPA cDNA clone and a functional dihydrofolate reductase clone subcloned into the unique *Clal* site. Stable transfectants were isolated by selection in growth medium without nucleosides for about 2 weeks. The transfected DNA containing the EPA sequence was amplified by repeated stepwise selection in methotrexate for ~2 months. To determine the levels of recombinant EPA protein synthesized, supernatants from transiently infected COS cells and stably infected CHO cells were analysed in a radioimmunoassay for EPA (J.C.G., J. Chan and D.W.G., in preparation). Samples which were positive for immunoreactive EPA were assayed for biological activity, by measuring stimulation of the growth of human peripheral blood BFU-E in methylcellulose culture³. Peripheral blood leukocytes were plated at a density of 3 × 10⁵ cells ml⁻¹ in 96-well tissue culture trays. Each well contained 0.1 ml of cells in methylcellulose and 0.01 ml of a dilution of the test supernatant. Control wells were those to which diluent alone was added, and they contained 20 colonies per well in panel *a* and 16 colonies per well in panel *b*.

(unpublished results). Treatment of U-937 (Fig. 2, lane 1) and Mo (lane 3) cells with the glucocorticoid dexamethasone appears to decrease the level of EPA mRNA by approximately twofold. Similar results have been seen with other T-cell-derived growth factors including IL-2¹¹ and granulocyte-monocyte colony-stimulating factor (GM-CSF)¹². Treatment of Mo cells with phytohaemagglutinin and phorbol myristate acetate (Fig. 2, lane 5) does not greatly increase the abundance of EPA mRNA over that produced constitutively. In similar conditions, GM-CSF mRNA levels are increased approximately two- to fourfold¹².

To provide definitive evidence that the cDNA clone encoded EPA, it was expressed in mammalian cells. The EPA cDNA

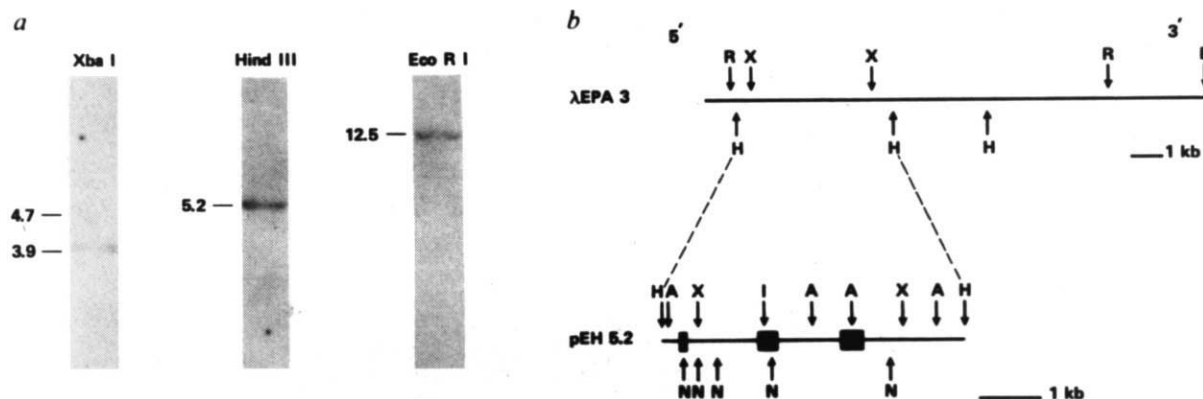


Fig. 4 Genomic organization of human EPA. *a*, Human DNA isolated from the HTLV-II-transformed J-LB-II T-lymphoblast cell line was digested with *Xba*I, *Hind*III and *Eco*RI and hybridized with the EPA cDNA. *b*, Restriction map of an EPA genomic clone in Charon 30 (λ EPA 3) and subcloned into pBR322 (pEH 5.2) are shown. Restriction sites are: R, *Eco*RI; X, *Xba*I; H, *Hind*III; A, *Ava*I; N, *Nco*I; I, *Hinc*II. **Methods.** High M_r DNA was prepared from a variety of cell lines, using standard procedures. The lengths of restriction fragments containing EPA sequences were determined by the method of Southern¹². A λ phage recombinant library was constructed using Charon 30 and DNA from the J-LB-III human T-lymphoblast cell line²⁰. The J-LB-III DNA was digested with the restriction enzyme *Sau*3A for various times and 10–25-kb fragments were isolated using a NaCl gradient²¹. The J-LB-III DNA was ligated to Charon 30 DNA which had been digested with *Bam*HI and purified on a NaCl gradient. Recombinant phage were recovered by *in vitro* packaging²². The recombinant phage were screened by hybridization to a probe consisting of a full-length EPA cDNA clone. Two smaller fragments of the EPA cDNA clone were isolated using *Pst*I, gel purified and used to generate detailed restriction maps of the EPA genomic clones.

clone was inserted into the 91023(B) mammalian expression vector and transiently expressed in COS monkey cells¹³. The concentration of EPA in the unfractionated COS cell supernatants was estimated, by radioimmunoassay, to be approximately $1 \mu\text{g ml}^{-1}$ 72 h after transfection, indicating efficient expression of the cDNA clone.

Supernatants from COS cells transiently infected with either the 91023(B) vector alone or the vector containing the EPA coding region were assayed for stimulation of human peripheral blood BFU-E. The results (Fig. 3a) demonstrate biological activity of the recombinant EPA transiently expressed in COS monkey cells. Good stimulation of erythroid bursts (>130% of control) is seen at concentrations as low as $\sim 5 \text{ pM}$ (based on the concentration of immunoreactive EPA). In every case, the supernatant from the mock-infected or uninfected COS cells had a slight stimulatory activity on human BFU-E, apparently due to some constitutive production by COS cells of a factor that stimulates human erythroid precursors.

In addition to transient expression of EPA in COS cells, it is useful to generate cell lines which constitutively produce high levels of EPA protein. Chinese hamster ovary cells with integrated copies of the 91023(B) vector containing the EPA coding region along with the dihydrofolate reductase gene were selected in methotrexate. After approximately 2 months of selection, levels of EPA protein in supernatants from 31 CHO cell clones were estimated by radioimmunoassay. One clone was found to constitutively produce levels of recombinant EPA similar to those obtained by transient expression in COS cells. The biological activity of recombinant EPA in the medium conditioned by this cell line was compared with medium conditioned by CHO cells which had been treated in the same way, but were not producing recombinant EPA. Figure 3b shows the stimulation of human peripheral blood BFU-E by recombinant EPA produced by this stable line of CHO cells. No endogenous burst-promoting activity is seen in the supernatant from CHO cells not producing recombinant EPA. The recombinant EPA is active in the picomolar range of concentrations, as is the purified protein from Mo-conditioned medium³.

To study the molecular organization of the human EPA gene sequences, high- M_r DNA was prepared from human T-cell lines (J-LB-II, J-LB-III, J-WM-IV, Mo), B-cell lines (Wil), a myeloid line (HL-60) and a fibroblast line (143) and analysed by the method of Southern¹⁴ for hybridization to the EPA cDNA clone. In all cases the same pattern was seen (Fig. 4a). The restriction enzyme *Xba*I generated a strongly hybridizing fragment of 3.9 kb and a faint band at 4.7 kb. A single fragment of 5.2 kb

and 12.5 kb was detected following digestion with *Hind*III and *Eco*RI respectively.

To obtain a more detailed map of the structure of the EPA gene, a recombinant λ phage library (prepared from Charon 30¹⁵ and J-LB-III DNA) was screened with the EPA cDNA clone as a probe. Three overlapping genomic EPA clones were obtained from approximately one million recombinant phage. The restriction map of one representative clone, λ EPA 3, is shown in Fig. 4b. This clone contains the 12.5-kb *Eco*RI fragment and the 5.2-kb *Hind*III fragment which was subcloned into pBR322 for further analysis (pEH 5.2). Restriction analysis of pEH 5.2 revealed that EPA appears to be encoded by a single gene approximately 3 kb in length, interrupted by at least two intervening sequences.

Human EPA specifically stimulates the growth of peripheral blood- and bone marrow-derived erythroid precursors from mouse and man. IL-3 is a 28,000- M_r glycoprotein isolated from the EL-4 mouse lymphoma cell line and WEHI-3 mouse myelomonocytic cells. Unlike EPA, IL-3 stimulates the growth of all haematopoietic precursors, including BFU-E⁶. Murine IL-3 has been molecularly cloned and sequenced^{16,17}. Comparison of the murine IL-3 sequence with the human EPA sequence, described here, reveals no homology. Thus EPA is not the human counterpart of murine IL-3, consistent with the biological observations that EPA does not stimulate pluripotent or myeloid precursor cells^{3,4}.

A computer search for protein homology did not reveal previously described proteins homologous to EPA (R. Doolittle, personal communication). A computer search for DNA homology (Genbank) did, however, reveal that a highly homologous partial murine cDNA clone had been isolated. This clone was obtained from virally induced C243 cells using hybrid selection of mRNAs whose translation products have antiviral interferon activity¹⁸. The cDNA clone pMIF 3/10 contains about 290 base pairs that could encode that 3' 39 amino acids of a protein plus the 3'-untranslated region. This cDNA clone was reported to encode a murine β -interferon-like protein, but the relationship between this molecule and EPA is unclear. Erythroid-potentiating activity has no interferon activity (our unpublished observation), and full-length cDNA clones of pMIF 3/10 have not been isolated and expressed to provide direct evidence for interferon activity.

The physiological functions of EPA *in vivo* are unknown. Since EPA stimulates murine erythroid precursors *in vitro*, as well as human, it should be possible to study *in vivo* effects of this modulator in the mouse. The availability of adequate quan-

titles of recombinant protein will be critical in elucidating the role of EPA in normal haematopoiesis. Additionally, the availability of cDNA and genomic clones will make it possible to study the evolution and expression of this interesting erythroid haematopoietic growth factor.

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Myxococcus xanthus spore coat protein S may have a similar structure to vertebrate lens $\beta\gamma$ -crystallins

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The Gram-negative bacterium *Myxococcus xanthus* has a complex life cycle¹ during which large amounts of a protein of relative molecular mass (M_r) 19,000, known as protein S, are assembled into a spore surface coat by a process that specifically requires calcium ions^{2,3}. The gene for protein S has been cloned and the DNA sequence shows that the gene product is composed of four internally repeated homologous sequences, each 40 amino acids long⁴. Although protein S resembles calmodulin both in its internally duplicated structure and its ability to bind calcium⁴, it apparently has a β -sheet secondary structure⁵ rather than the helix-loop-helix motifs that characterize the calmodulin family^{6–8}. We now show that protein S has a striking homology with the β - and γ -crystallins of the vertebrate eye lens^{9–11} which are β -sheet proteins with internally duplicated structures. This implies that the β - and γ -crystallins evolved from already existing proteins, whose ancestors occurred in the prokaryotes. The biological function of protein S, as a closely packed, stable protein in a relatively dehydrated environment, has implications for the functions of crystallins, which are found closely packed in the lens fibre cells, where their stability is essential for maintenance of transparency.

γ -Crystallin has an internally repeated sequence of ~40 amino acids^{9–11} arranged as two globular domains related by a pseudo-dyad. Each domain is formed from two Greek key motifs

(strands $dabc$) related by further pseudo-dyads and arranged as two antiparallel β -sheets, each sheet composed of strands $c'dab$ where strand c' is contributed by one motif and dab by the second. In motif 1 the characteristic fold is made possible by the presence of a glycine at position 13 which allows the β -hairpin formed by strands a and b to fold back on to the sheet so that the peptide nitrogen of residue 11 hydrogen-bonds to the buried side chain of serine 34 which just precedes strand d . This leads to interactions between the aromatic amino acids at residues 6 and 11, which are protected from solvent by an arginine at residue 36. This arrangement is repeated in the other motifs with a small variation in sheet 3 only¹⁰. The amino-acid sequences of the β - and γ -crystallins from several species^{12–19} have this arrangement of residues in common, suggesting an evolutionary relationship between the two crystallin classes and the existence of a protein structural superfamily, the $\beta\gamma$ -crystallins¹⁷.

Analysis of the repeated sequences of bovine γ -crystallin II (γ_{II}) shows that motifs γ_1 and γ_3 , and motifs γ_2 and γ_4 have the strongest homologies. Motifs γ_2 and γ_4 are longer than γ_1 and γ_3 , and appear to have several residues inserted in the loop region between strands c and d of the Greek key motifs. A similar analysis of protein S shows that S1 and S3, and S2 and S4 are the pairs with the highest homologies (60% and 45%), indicating a similar pattern of duplication, but greater similarity between the two halves than in $\beta\gamma$ -crystallins. The optimum correspondence of repeated sequences between protein S and γ_{II} is nonlinear (Fig. 1). In terms of the number of residues, the S2/S4 pair corresponds most closely to motif γ_1 of γ -crystallin II. Similarly, S1/S3 corresponds most closely to motifs γ_2 and (not shown) γ_4 . At the level of sequence identity also, S2/S4 most closely resembles γ_1 , with 33% and 23% identity respectively, whereas S1/S3 matches γ_2/γ_4 , with 26% identity between S1 and γ_2 and 19% identity between S3 and γ_4 . The N-terminal domain of γ_{II} ($\gamma_1\gamma_2$) was used as the basis for a model for both proposed domains of protein S. Figure 2 shows a model structure for S1S2, the N-terminal domain of protein S. The structurally important residues are all present, allowing the main chain to follow the same path, forming the characteristic folded hairpin structure described for γ (refs 9, 10). The

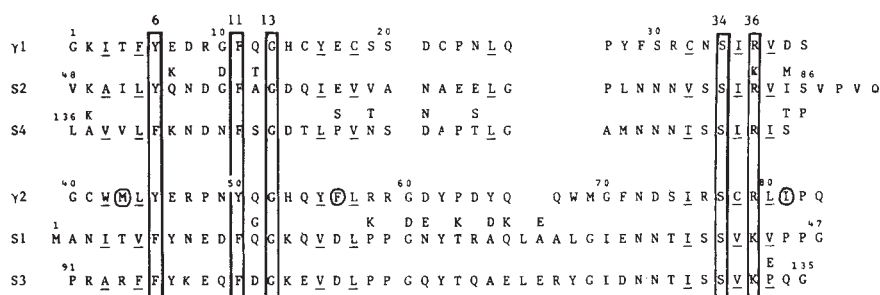
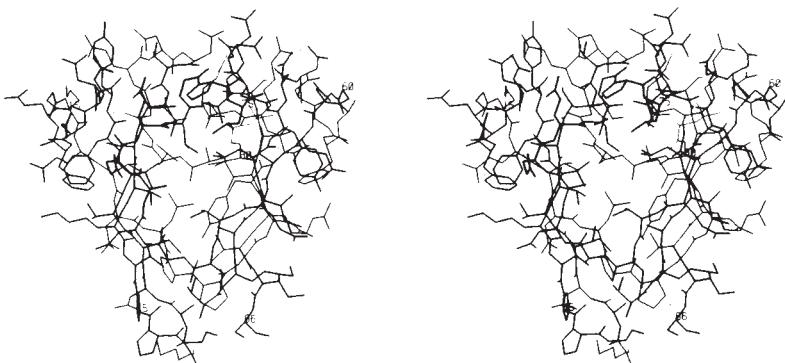


Fig. 1 Alignment of the four repeated sequences of protein S (S1–S4) with the first two motifs of γ -crystallin II (γ_1 , γ_2). The protein S sequence is that defined for gene 2, with the residues for gene 1 given above; there is an inserted alanine between Ser 146 and Gly 147 in gene 1.

Fig. 2 A stereo view of the molecular model of one domain (S1 + S2) of protein S. The model was constructed by first aligning the sequences of protein S and bovine γ_{11} so that the structurally important glycine and serine residues coincide. The alignments are shown in Fig. 1. To simplify the comparison, only one motif of each pair in γ_{11} is shown. The two domains were modelled using the N-terminal domain of γ_{11} (comprising motifs $\gamma 1\gamma 2$) as a guide, with γ_{11} coordinates taken from the structure refined at 1.6 Å resolution¹¹. The interactive graphics program FRODO (ref. 25, modified by T. A. Jones and I. J. Tickle) run on an Evans and Sutherland PS2 was used for model-building in the manner described previously for bovine β Bp-crystallin²⁶ and murine $\beta 23$ -crystallin¹⁸. Main-chain atoms were not moved, except where two insertions were required in a surface loop of $\gamma 2$ to model S1 and S3. The coordinates of invariant side chains were retained. For conservative changes, side-chain conformations were modelled closely on the crystallographic coordinates; remaining side chains were modelled to optimize favourable interactions and to avoid disallowed non-bonded contacts. The models have been subjected to energy minimization.



extensive hydrogen-bonding in the β -sheets and elsewhere¹⁰ is also preserved.

Because of the non-sequential correspondence between related motifs, it was necessary to make and break inter-motif connections in the γ_{11} structure; this is easily achieved as each domain contains a pseudo 2-fold axis, the N-terminus of each strand *a* and the C-terminus of each opposing strand *d'* being disposed in space in a similar way (Fig. 3). We have attempted to build a model with sequential alignment of the two polypeptides, but it gives less good packing of the hydrophobic core.

The model shown in Fig. 2 indicates that the residues which occupy the space between the two sheets in each domain (S1S2 or S3S4) of protein S are conserved as hydrophobic, although there is a small overall decrease in their volume (see Fig. 1). For example, Tyr 16 of $\gamma 1$ and the topologically equivalent Tyr 55 of $\gamma 2$ which lie on strand *c* at the edge of the sheet are replaced by leucine, valine or isoleucine in protein S. More importantly, the buried Trp 42 is replaced by isoleucine in S1 and an alanine in S3. However, the inserted residues in the loop between strands *c* and *d* in S1 and S3 of protein S appear to contribute to the core packing, and Gly 70 of $\gamma 2$ is replaced by isoleucine in protein S. Molecular mechanics calculations imply that the two β -sheets are packed more closely in protein S than in γ -crystallin II, while preserving the hydrophobicity of the contacts. Similar small relative shifts in β -sheet positions have been shown to occur in the azurin/plastocyanin family²⁰. The exact relationship of the two sheets must await a detailed X-ray analysis, which is now underway²¹.

In β -crystallins the two domains ($\gamma 1\gamma 2$ and $\gamma 3\gamma 4$) are related by a further pseudo-dyad which gives rise to closely packed hydrophobic interactions between residues of strands *a*, *b* and *d* of $\gamma 2$ and $\gamma 4$, involving residues which are conserved as hydrophobic in all $\beta\gamma$ -crystallins. In motifs S1 and S3 of protein S, these are replaced by several charged groups, indicating hydrophilic contacts with solvent. However, inter-domain contacts may instead be formed between the sheets that are mainly composed of motifs S2 and S4, where hydrophobic residues do occur. Most of the surface residues of protein S are hydrophilic and many are charged, although the unusually large number of ion pairs found in γ -crystallin II, for example, Arg 36 to Asp 61 (refs 9, 10), are absent from protein S. Protein S is more acidic than γ -crystallin II, mainly because of the smaller number of arginines/lysines rather than an increase in the number of acidic residues. As in γ -crystallin II, there is a predominance of aspartates over glutamates.

Overall, the proposed structure for protein S is consistent with the data presented by Inouye *et al.*^{4,5}. In particular, the inter-domain connecting peptide, as in bovine γ_{11} , would be relatively exposed and potentially vulnerable to tryptic cleavage (in the case of protein S). The so-called 'tryptic core structure'¹⁴ would thus correspond to the first domain of protein S.

Calcium ions are thought to be specifically involved in binding

protein S to spores. The existence of layers of protein S which are 300 Å thick² indicates that there may be intermolecular interactions between protein S molecules (maximum dimension ~80 Å) as well as with specific receptors on the spore. Because identical crystals of protein S may be obtained with or without calcium^{5,21} and as calcium binding has no significant effects on the circular dichroism of protein S⁵, protein S may be crosslinked to its receptors on the spore and possibly to other protein S molecules directly through surface Ca^{2+} sites which do not affect the conformation of the molecule.

As there is no direct evidence implicating any particular residues in calcium binding, we began by searching the model surfaces of the protein S gene 1 and 2 products for conserved arrangements of oxygen ligands, for example carboxylates of aspartate or glutamate, amides of asparagine or glutamine, O γ of serine or threonine, and peptide carbonyl oxygens. Such calcium-binding ligands have been identified in a wide range of proteins. The most obvious candidate is a surface group of four acidic residues found close to the folded β -hairpin structure and including residues Glu 10, Glu 70, Glu 71 and Asp 11 (see Fig. 4); this would give only one binding site for calcium per protein S molecule and would be consistent with the ability of only ~45% of the 'tryptic core structures' to self-assemble on the protein S-deficient spores, as only one of the two domains would bind calcium. An alternative binding site occurs in each of the sequences S1 to S4 and therefore occurs twice in each domain. This binding site is composed of the conserved topological equivalent residues Glu 10, Glu 99, Asp 56 and Asp 144. The other ligands at each of these putative calcium-binding sites are derived from the conservatively varied sequence Glu 35-Asn 36-Asn 37-Thr 38-Ile 39-Ser 40 and equivalents of this, represent-

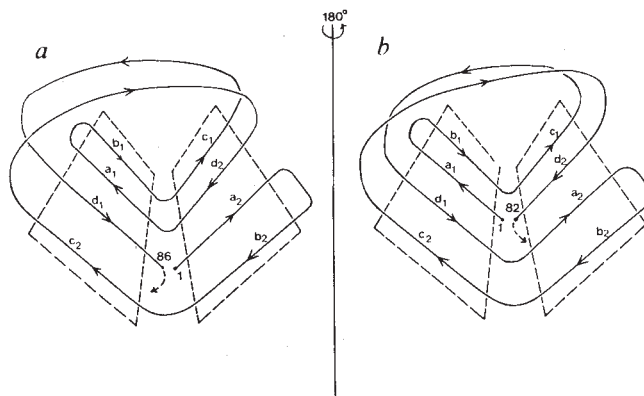
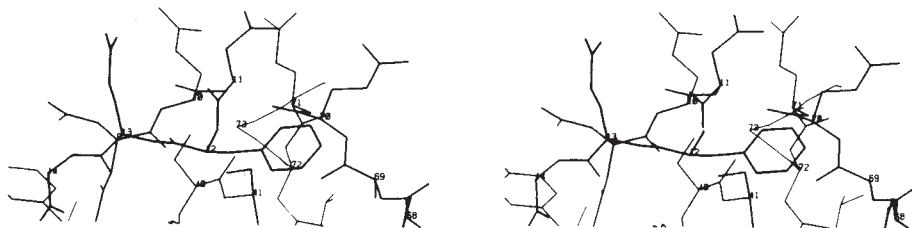


Fig. 3 The suggested arrangement of the strands in the first two repeated sequences (S1 and S2) of protein S (a) compared with that found in γ -crystallin II (b).

Fig. 4 A stereo view of a possible calcium-binding site involving Glu 10, Asp 11, Glu 70 and Glu 71.



ing part of the sequence Glu 35 to Lys 43 which has been identified by Inouye *et al.*²² as homologous to the calcium-binding sequence of calmodulin. However, if our model is correct, the binding cannot involve Ser 41, as this residue is buried. There is no residue in protein S corresponding to the final glutamate which is conserved in the loop region of all members of the calmodulin family. These sites are the most obvious candidates for surface Ca^{2+} binding.

Finally, we must consider the implications of the observed structural similarity between protein S and $\beta\gamma$ -crystallins for the function and evolution of vertebrate lens proteins. The putative calcium-binding sites described above are not generally conserved in bovine γ_{11} or in other $\beta\gamma$ -crystallins¹²⁻¹⁹. However, calcium ions do seem to have an effect on the aggregation of crystallin in certain systems²³. Lens proteins have a short-range order which leads to slowly changing protein concentrations and a smooth change of refractive index through the transparent lens; conceivably, this could be influenced by calcium, suggesting a specific role for ions in certain forms of cataract.

γ -Crystallins and protein S are highly stable molecules²⁴; this may be due to the closely hydrogen-bonded structure of the repeated Greek key motifs, which lack the loose external loops found in many proteins, and to their symmetrical, tightly packed arrangements. Thermodynamic stability may be a general consequence of protein structures developed to survive for long periods in relatively dehydrated environments without turnover; this would apply to both spore proteins and lens crystallins.

γ -Crystallins have high proportion of cysteine and aromatic residues which are not found in protein S. None of the six cysteines found in γ_{12} is retained in protein S. The two tryptophans in γ -crystallin are replaced by aliphatic residues and the loss of 10 phenylalanines and tyrosines is compensated for by only three at other positions. It has been argued^{10,11} that the cysteines and associated aromatic amino acids are important in protecting the lens proteins from the effects of ultraviolet radiation and other abuses which lead to free radical formation. These aspects of the $\beta\gamma$ -crystallin structure may have evolved to meet the special requirements of the lens.

The intramolecular homology of protein S indicates that a pattern of gene duplication similar to that proposed for $\beta\gamma$ -crystallins has occurred. The fact that S2 resembles γ_1 and S1 resembles γ_2 suggests that a common ancestor could have been an asymmetrical dimer of two 40-residue units. In the crystallin line of descent, the genes for these molecules were fused in the order γ_1 - γ_2 while the reverse order occurred for protein S. In both cases, further duplication and fusion led to the present two-domain structures.

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The nucleotide sequences of *copia* and *copia*-related RNA in *Drosophila* virus-like particles

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We have shown previously that *Drosophila* cells contain virus-like particles (VLPs) containing 5-kilobase (kb) RNA that hybridizes to a transposable element, termed *copia*. We have suggested that VLPs and *copia* are derivatives of viral particles and proviral forms, respectively, of 'copia' retrovirus, a putative *Drosophila* retrovirus¹. To further clarify the relationship between *copia* and *copia*-related RNA in VLPs (VLP H-RNA), we determined and compared their nucleotide sequences. VLP H-RNA was found to be an unspliced, genome-sized transcript of *copia*, and, like retroviral genome RNA, VLP H-RNA is terminally redundant with termini localized in the long terminal repeats (LTRs) of *copia*. VLP H-RNA contains two long open reading frames (ORFs), one of which includes the coding sequence for a predominant VLP protein of relative molecular mass (M_r) 31,000 (31K). Here we show that, in contrast to 17.6 ORF2 (ref. 2), ORFs of *copia* have no extensive amino-acid sequence homology to the RT region² of the reverse transcriptase of retrovirus in vertebrates. Because of a one-base insertion/deletion, the two ORFs in VLP H-RNA are fused and become a single, longer ORF in a genomic *copia*.

Using cDm2055 DNA³ as a probe, we isolated 20 recombinant phage containing *copia* from a cloned library of *Drosophila melanogaster* DNA⁴ and made restriction maps (data not

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Figure 1 consists of two panels, A and B, showing schematic representations of experimental designs. Panel A shows a sequence of events: a box labeled 'a' (15s), followed by a sequence of 'y' (6s), 'a' (15s), 'l' (15s), 'e' (15s), 'e' (15s), and 't' (22s), leading to a box labeled 'p' (21s). Panel B shows a sequence of events: a box labeled 'Capacitance' (10s), followed by a sequence of 'l' (10s), 'e' (10s), 'e' (10s), and 't' (10s), leading to a box labeled 'p' (10s).

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Fig. 1 (Opposite) Nucleotide sequences of *copia* and cDNA of *copia*-related RNA in VLP (VLP H-RNA). The complete nucleotide sequence of *copia* in λ cop88 is shown as the putative sense strand in L1, whereas L3 and L3', respectively, show the composite cDNA nucleotide sequence of VLP H-RNA and the DNA sequence complementary to the 5' terminal sequence of VLP H-RNA determined by the dideoxy-chain termination method using VLP H-RNA as a template. The ranges of long ORFs found in both *copia* and VLP H-RNA are shown by L-shaped arrows labelled ORF, with amino-acid sequences shown below the corresponding nucleotide sequences (L2 and L4). Dashes, nucleotide or amino-acid sequences identical to those of *copia* in L1 and L2. Os in L3 indicate one-nucleotide deletions in VLP H-RNA, whereas # denotes the termination codon of each ORF. Underlines, nucleotide sequences corresponding to the duplicated target DNA; a and i, 5' start sites of cellular 2- and 5-kb RNA⁶; b, expected polyadenylation signal; c, e, h, nucleotide sequence complementary to the 26-nucleotide long synthetic oligodeoxynucleotide for primer extension experiments; d, the amino-acid sequence best conserved among various polymerases^{10,11}; f, polypurine sequence corresponding to the counterparts of other *Drosophila* transposons and vertebrate retroviruses (g)¹². Overlines labelled w and y, respectively, show the 5' terminal region of VLP H-RNA in the 5' LTR and its equivalent in the 3' LTR. Two vertical arrows indicate 5' ends of two major VLP H-RNAs. Thin lines associated with the arrows show the degree of inaccuracy (± 1 nucleotide) for the determination of 5' ends. Overlines z and x exhibit the poly(A) attachment site of VLP H-RNA in the 3' LTR and its equivalent in the 5' LTR, respectively. The ranges of 5' LTR, 3' LTR and expected coding region of the predominant M, 31,000 VLP protein (31K) are shown by L-shaped arrows. The end of the 31K region was tentatively determined based on the relative molecular mass and amino-acid composition of the 31K VLP protein. The base substitutions and deletion found in the nucleotide sequences of *copia* regions 1-1,143 and 2,302-2,931 (described in refs 6, 7) are shown by small letters and o, respectively, above the *copia* sequence (L1).

Methods. The nucleotide sequence of *copia* was determined by digesting DNA extracted from λ cop88 by various restriction enzymes and recloning the resultant DNA segments containing *copia* pieces using plasmid pUC9 (ref. 13) as a cloning vector. Sizes and locations of the cloned DNA segments are shown by thick lines with arrows and numbers in scheme A. After *Escherichia coli* cells (JM83 (ref. 13) harbouring a desired plasmid) were cultured in 5–10 ml L-broth for 12–15 h at 37 °C with shaking, plasmid DNA was prepared by the alkali method¹⁴. Contaminated RNA was removed by a treatment of 10 μ g ml⁻¹ of RNase for 30 min at 37 °C and final concentration of 7.5% polyethylene glycol in 0.94 M NaCl for 1 h at 0 °C. Nucleotide sequence was determined by the chain termination method¹⁵ modified by M. Hattori (unpublished) using the DNA cloned in pUC9. Two different kinds of synthetic oligonucleotides [M13 single-stranded primer (5' GTAAACGACGCGCCAGT3') and M13 reverse sequence primer (5' CAG-GAAACAGCTATGAC3') (P-L Biochemicals)] were used as primers for sequencing. The overall strategy for sequencing is depicted by thin underlines in the lower margin illustration (A). Scale bar; 1,000 bp; the two boxes show the LTRs. Restriction enzyme cutting sites used are as follows: a, *AccI*; b, *Bam*HI; c, *Clal*; e, *Eco*RI; h, *Hind*III; i, *Bal*I; p, *Hpa*I; s, *Pst*I; t, *Aat*I; v, *Pvu*II; x, *Xba*I. The nucleotide sequence of VLP H-RNA was determined as follows. As described previously¹, VLP was purified from a 5 l culture of GM₂ cells and total VLP RNA extracted. After the VLP H-RNA was purified by gel electrophoresis, cDNA was constructed and cloned into pBR322 using the procedures described by Maniatis *et al.*¹⁶. Restriction mapping and blot hybridization allowed overlapping of the cDNA inserts obtained and a composite restriction map of the cDNA of VLP H-RNA to be made. The restriction map obtained was found to be very similar to that of *copia*. Thus, in scheme B, their relative positions are depicted using the restriction map of the *copia* in λ cop88 as a reference. Plasmid DNA was purified essentially as described elsewhere². The complete or partial nucleotide sequences of A-25, G-19, G-29, G-34, G-46, J-15, J-16, J-19, H-15 and 3-5 were determined by the procedures of Maxam and Gilbert¹⁷. We found no discrepancy in the nucleotide sequence in the overlapped region. The position of poly(A) attachment site in G-29 was identical to that in 3-5. Thus, in L3, the composite sequence is shown as the nucleotide sequence of the cDNA of VLP H-RNA. We consider the sequence shown in L3 to be identical to that of VLP H-RNA, apart from the DNA-RNA difference, because (1) at its 3' end, it contains a long poly(A) tract, a typical terminal structure of eukaryotic mRNA and retroviral genome RNA; and (2) a separate experiment (not shown) indicated that only the DNA strand of *copia*, complementary to the one whose sequence is shown in L1, is hybridizable to VLP H-RNA. The nucleotide sequence near the 5' terminus of VLP H-RNA was determined by the chain termination method¹⁵. After 3 μ g VLP RNA and 65 ng of ³²P-labelled oligodeoxynucleotide (see Fig. 4a) in 40 μ l 0.1 M NaCl, 1 mM EDTA and 10 mM Tris-HCl (pH 8.3) was preincubated at 85 °C for 5 min, then at 60 °C for 10 min. 10 μ l 500 mM Tris-HCl (pH 8.3) containing 50 mM dithiothreitol and 80 mM MgCl₂, 1 μ l RNasin, 1 μ l reverse transcriptase and 8 μ l 25 μ M dCTP were added. To each of four 14- μ l aliquots, 8 μ l of the dNTP-dNTP mixture of M13 sequencing kits (Takara Shuzo Ltd, Japan) was added. The resultant mixture was then incubated at 40 °C for 20 min. After four dNTPs (1 mM each) in 4 μ l was added, the incubation was continued for further 20 min. The reaction products were precipitated by adding 2.5 μ l 3 M sodium acetate (pH 5.2) and 2 vol ethyl alcohol, and subjected to gel electrophoresis for sequencing.

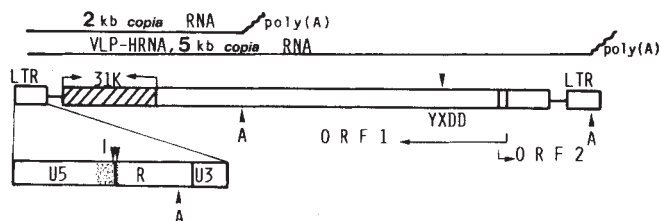


Fig. 2 Genome organization of *copia* and its gene products shown schematically. The 5' LTR has been enlarged to make the internal structure of LTR more easily discernible. The ambiguity in the 5' end of U3 results from the three consecutive A residues at the poly(A) attachment site, whereas that of R results from the real heterogeneity in the 5' end of VLP H-RNA. Two downward arrows labelled I show the positions of the 5' ends of the two major VLP H-RNAs. The locations of the putative polyadenylation signal of *copia*, AAATAATAATC, are shown by the arrows labelled A. Note that one of the polyadenylation signals is near the region corresponding to the 3' end of 2-kb *copia* RNA. The structure of genomic *copia* is based on the sequence of VLP H-RNA, although a *copia* in λ cop88 contains a single ORF covering the entire regions of both ORFs 1 and 2 (see Fig. 1). A polypurine tract similar to those of other *Drosophila* transposons and vertebrate retroviruses¹² can be seen in the 3'-untranslated region. The expected coding sequence of the predominant 31K VLP protein is shown by a shaded box and appears to be included in both 2- and 5-kb *copia* RNA (VLP H-RNA). The location of the most conservative amino-acid sequence among RNA dependent polymerases, Y(M, L, V or G)DD(hydrophobic amino acid)₃, is shown by the arrow at YXDD. Association of 31K VLP protein and VLP H-RNA is considered to result in the production of VLP.

shown). As the restriction map of the *copia* in λ cop88 was very similar to that of most of the *copias* that we cloned, the complete nucleotide sequence of the *copia* in λ cop88 was determined as representative of *copia* sequences (Fig. 1, L1). The *copia* in λ cop88 is 5,143 base pairs (bp) in length and terminates with 276-bp LTRs whose nucleotide sequences are identical or very similar to the published LTR sequences of other *copias*⁵. Partial nucleotide sequences of the region flanked by LTRs have been reported elsewhere^{6,7}. The sequences in these studies are essentially identical to the counterparts described here (Fig. 1). Translation of the nucleotide sequence showed that *copia* contains a long ORF, ORFg (Fig. 1, L2), residing on a DNA strand corresponding to the major cellular *copia* RNAs⁶.

We purified VLP H-RNA by gel electrophoresis and made complementary DNA clones. We found no clones that completely covered the entire region of the VLP H-RNA; the information from the 5' end of the RNA could not be recovered because S₁ nuclease was used. Nevertheless, the overlapping of several cDNA clones (Fig. 1) allowed us to determine 97% of the total sequence of VLP H-RNA from its 3' end (Fig. 1, L3). The nucleotide sequence of the major portion of the residual 3% was determined by the chain termination method using VLP H-RNA as a template (Fig. 1, L3'). These results show that the nucleotide sequence of VLP H-RNA corresponds to the *copia* sequence shown in Fig. 1 (L1) except for some minor base changes and a terminal poly(A) tract. Because of a deletion of an A residue located somewhere between residues 4,220 and 4,224, the ORF of *copia* (ORFg) is split in two, slightly overlapping ORFs 1 and 2 in VLP H-RNA (Fig. 1). The 3' end of VLP H-RNA that lacks poly(A) is located in the 3' LTR of *copia*. However, we found no AATAAA, a typical signal for polyadenylation, in this LTR. The sequence AAATATAATC is present ~20 nucleotides upstream from the poly(A) attachment site of the 5-kb RNA and in the region expected for the 3' terminus of the 2-kb RNA (ref. 6; Figs 1, 2). This sequence, or part of it, may function as a polyadenylation signal in *copia*.

To determine the location of the 5' end of VLP H-RNA, we carried out a primer extension experiment using total VLP RNA and ³²P-labelled, 26 nucleotide long synthetic oligodeoxynucleo-

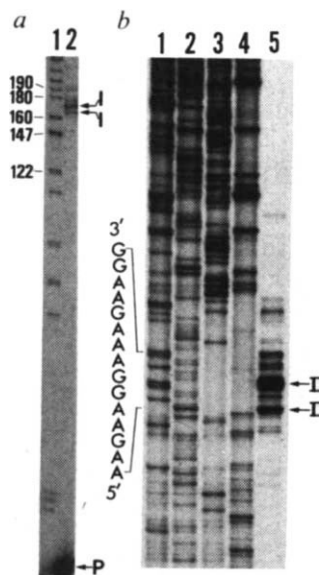


Fig. 3 Determination of the 5' end of VLP H-RNA by primer extension. The expected locations of the 5' ends of two major VLP H-RNAs are shown by arrows labelled I, whereas the arrow labelled P shows that of the 26-nucleotide-long primer used. *a*, pBR322 DNA was digested with *Hpa*II, labelled with 32 P by kinase treatment, denatured and used as a marker. Molecular sizes in the left margin are in bp. Using total VLP RNA (3 μ g) and 26-nucleotide-long synthetic DNA (65 ng) (see Fig. 4a) as a template and primer, a primer extension experiment was carried out essentially according to Treisman *et al.*¹⁸. After the reaction, samples were denatured, applied to 45-cm-long 8% polyacrylamide gel in the presence of 7 M urea, and electrophoresed. Lane 1, pBR322 marker; lane 2, products of primer extension of VLP RNA with primer. *b*, Numbers in lanes 1-4 show the nucleotide sequence pattern obtained by the modified dideoxy-chain termination method when the DNA of plasmid 15 (see scheme *a* in Fig. 1) and the 26-nucleotide-long synthetic DNA identical to that used for primer extension of VLP RNA (Fig. 4a) were used as a template and primer. Lanes 1, 2, 3 and 4, respectively, show the locations of G, A, T and C residues. The sample in lane 5 is an aliquot of the reaction products shown in lane 2 in *a*.

tide (see Fig. 4a). Two intensive bands, 166 and 172 nucleotides in length, along with ~20 less intensive bands of similar size, were observed (Fig. 3), suggesting that VLP H-RNA is heterogeneous in the location of its 5' end. By comparing the positions of these bands (Fig. 3b, lane 5) with the dideoxy-chain termination pattern of the 5' terminal region of *copia* (lanes 1-4), we determined the range of the 5' ends of VLP H-RNA (Fig. 1, overline *w*). According to Flavell *et al.*⁶, the 5' ends of cellular *copia* RNA are similarly heterogeneous in sequence and localized in an almost identical region in the 5' LTR of *copia* (see Fig. 1). Taking together their findings and the results described above, we conclude that VLP H-RNA is an unspliced genome-sized transcript of *copia* (see Fig. 2). As with vertebrate retrovirus, VLP H-RNA has terminal redundancy, 77-116 nucleotides in length, and the *copia* LTR can be divided into three regions, 106-142 bp (U5), 77-116 bp (R) and 54-57 bp (U3) (Fig. 2). These findings provide additional evidence for the notion that *copia* RNA may be reverse-transcribable^{1,8}.

VLP H-RNA has weak messenger RNA activity and can be translated into polypeptides immunologically related to VLP proteins¹. To identify the coding region for the predominant 31K VLP protein¹, the partial amino-acid sequence of one of the polypeptides obtained from the 31K protein was determined and found to be identical to that of the amino-terminal region of the polypeptide expected to be derived from ORFg of *copia* (or ORF1 of VLP H-RNA) (Fig. 4b). Furthermore, the calculated amino-acid composition of the *M_r* 31,000 amino-terminal

a 5' ATTAGGCATGGACTGGGCCATAACC 3'

b 31K VLP protein: Asp-Lys-Ala-Lys-(X)-Asn-Ile-Lys-Pro-
copia ORF1 protein: Met-Asp-Lys-Ala-Lys-Arg-Asn-Ile-Lys-Pro-
 (Amino terminus)

Fig. 4 *a*, Nucleotide sequence of a 26-nucleotide-long synthetic oligonucleotide used as the primer. It is complementary to a portion of *copia* sequence (see underline *d* in Fig. 1, L1) and was synthesized using a Model 380A synthesizer (Applied Biosystems). *b*, Comparison of the partial amino-acid sequence of the predominant 31K protein of VLP and that of the amino-terminal region of the putative ORFg (or ORF1) protein of *copia*. X, an unidentified amino acid.

Methods. VLP was purified from a 12-l culture as described previously¹ and the predominant 31K protein purified by large-scale SDS-polyacrylamide gel electrophoresis. The 31K VLP protein was treated with cyanogen bromide¹⁹ and the resultant polypeptides separated and purified by reverse-phase liquid chromatography (RP300 (4.6 $\times$ 250 mm), Brown Lee). The partial amino-acid sequence of the major polypeptide was determined by stepwise Edman degradation using a gas-phase automated sequencer (Model 470 A, Applied Biosystems). The recovery of other polypeptides was low because of self-aggregation.

portion of the putative *copia* ORFg (ORF1) polypeptide was found to be almost identical to that observed for the 31K VLP protein (data not shown). Thus, we suggest that the coding region for the 31K VLP protein is located in the 5' terminal region of the ORFg (ORF1) (Figs 1, 2b) and the 31K VLP protein is one of the cleavage products of the putative polypeptide(s) expected to be produced from ORFg (or ORF1).

To determine whether *copia* has the coding sequence for the reverse-transcriptase-like enzyme associated with VLP¹, we compared the amino-acid sequences of ORFs 1 and 2 with those of the reverse transcriptases of retroviruses⁹ and equivalent polypeptides of *Drosophila* transposons such as 17.6 (ref. 2). However, no appreciable homology could be seen except for Tyr-(Met, Leu, Val or Gly)-Asp-Asp(hydrophobic amino acid)₃, the most conservative sequence among various polymerases^{10,11}, near the carboxy-terminus of the ORFg (ORF1) polypeptide(s) (Figs 1, 2). This suggests that *copia* contains the coding region for the reverse-transcriptase-like enzyme, which has a different amino-acid sequence from the counterparts of other *copia*-like elements² and retroviruses⁹. Furthermore, this reduced homology partly results from the extensive rearrangement within the putative *pol* region of *copia* (the region between nucleotides 1,242 and 4,658 in Fig. 1; K.S. *et al.*, unpublished).

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Phosphorylation of the glucose transporter *in vitro* and *in vivo* by protein kinase C

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The Ca^{2+} - and phospholipid-dependent protein kinase (protein kinase C) is present in many mammalian tissues¹, and its important physiological protein substrates are only now beginning to be identified. A useful advance in identifying these intracellular substrates has been the recognition that the kinase is the receptor for phorbol esters, which stimulate phosphotransferase activity²⁻⁴. Phorbol ester-induced changes in protein phosphorylation in intact cells may thus be taken, in part, as a probable indication of protein kinase C activation. The many cellular effects of phorbol esters include the stimulation of glucose uptake⁵⁻⁸, although the response of glucose uptake to phorbol esters appears to be complex, apparently varying in response time and requirement for protein synthesis^{6,7}. Such observations prompted us to explore one possible explanation for the alteration of glucose uptake, namely, phosphorylation of the glucose transporter by protein kinase C. We report here that incubation of purified human erythrocyte glucose transporter with rat brain protein kinase C results in the phosphorylation of a protein of relative molecular mass (M_r) 50,000–60,000 which has subsequently been identified as the glucose transporter by specific immunoprecipitation with a monoclonal antibody. Immunoprecipitation of membrane proteins from ^{32}P -labelled human erythrocytes revealed a phorbol ester-stimulated phosphorylation of the transporter. This covalent modification of the glucose transporter may thus, in part, underlie the ability of phorbol esters and certain hormones to stimulate glucose uptake.

Incubation of a preparation of purified human erythrocyte glucose transporter with protein kinase C resulted in the phosphorylation of a protein that migrated on SDS-gel electrophoresis as a broad band of apparent M_r 50,000–60,000 (50–60K), together with higher- M_r species, some of which did not enter the SDS gel (Fig. 1a). The phosphorylation was absolutely dependent on the addition of Ca^{2+} ; because the transporter preparation contained erythrocyte membrane phospholipids, added phosphatidylserine caused only a slight increase in phosphorylation. It has been shown previously that the transporter is a glycoprotein that migrates as a broad band of apparent M_r 50–60K on SDS gels, due to heterogeneity in its oligosaccharides^{9,10}. In addition, on incubation with detergents, the protein has a tendency to form dimers and higher- M_r aggregates which is not prevented by reducing agents^{10,11}. No phosphorylation of these bands was observed on incubation with casein kinase I, casein kinase II or the catalytic subunit of the cyclic AMP-dependent protein kinase (not shown). Because the preparation of glucose transporter used in these experiments was only 70–80% pure¹¹, the identity of the ^{32}P -labelled protein as the transporter was established unequivocally by specific immunoprecipitation with a monoclonal antibody directed against the erythrocyte transporter (antibody G3)¹² (Fig. 1b). The bands at $M_r \sim 100,000$ and at the top of the gel seen in Fig. 1b (and in Fig. 3b) are due to the dimer and the higher- M_r aggregates of the transporter^{10,11}.

Nearly 0.5 mol of ^{32}P -phosphate per mol cytochalasin B binding was incorporated into the transporter on prolonged incubation with protein kinase C (Fig. 2a). Although the purified

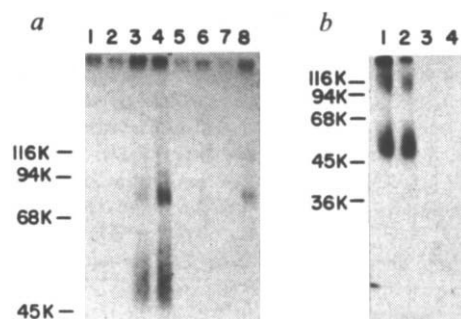


Fig. 1 *In vitro* phosphorylation of glucose transporter by protein kinase C (a) and ^{32}P -protein identification by immunoprecipitation (b). In a, human erythrocyte glucose transporter ($55 \mu\text{g ml}^{-1}$; lanes 1–4) or buffer (lanes 5–8) was incubated for 30 min with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ ($100 \mu\text{M}$), MgCl_2 (10 mM) and protein kinase C ($4,000 \text{ pmol units ml}^{-1}$) in the presence of EGTA (1 mM ; lanes 1, 5), EGTA plus phosphatidylserine (PS) ($40 \mu\text{g ml}^{-1}$; lanes 2, 6), CaCl_2 (1 mM ; lanes 3, 7), and CaCl_2 plus PS (lanes 4, 8). No phosphorylation of any protein was observed in the absence of added kinase. In b, aliquots of the incubations of the transporter with added kinase were immunoprecipitated with monoclonal antibody to the transporter (lanes 1, 2) or with control monoclonal antibody (lanes 3, 4), and the immunoprecipitates separated by SDS-gel electrophoresis before autoradiography. Lanes 1, 3, kinase reaction with CaCl_2 and PS; lanes 2, 4, kinase reaction with CaCl_2 alone. M_r markers are indicated on the left of a and b.

Methods. The glucose transporter from human erythrocytes was purified by the method of Baldwin *et al.*¹¹. Protein kinase C was partially purified from rat brain through the gel filtration step by the method of Kikkawa *et al.*¹; the kinase used in these experiments had a specific activity of 200 nmol per min per mg protein against histone III-S. Kinase assays were performed by the method of Parker *et al.*⁴. Immunoprecipitation was carried out by solubilization of the transporter ($1 \mu\text{g}$) in 1.5% octyl glucoside, followed by incubation of the samples on ice with 1.5% octyl glucoside/protein A-Sepharose to which $10 \mu\text{g}$ of the specific or control monoclonal IgG was complexed. The immune complexes were washed and then solubilized with 1.0% SDS in the absence of reducing agents and without heating. SDS gels were run according to Laemmli²⁴.

transporter is contained in largely unsealed membranes of erythrocyte lipids^{11,13}, it is possible that not all of the potential phosphorylation sites are exposed to the kinase or that the preparation already contains phosphorylated amino acids. Limited acid hydrolysis of ^{32}P -labelled transporter and subsequent thin-layer electrophoresis revealed only ^{32}P -phosphoserine (not shown). HPLC separation of ^{32}P -phosphopeptides, generated by prolonged tryptic digestion, yielded only one ^{32}P -peptide (Fig. 2b) which also contained only ^{32}P -phosphoserine. These data support the conclusion that the phosphorylation is confined to a single protein in the transporter preparation and probably to a limited number of phosphorylation sites.

To determine whether protein kinase C phosphorylates the glucose transporter *in vivo*, human erythrocytes were labelled with ^{32}P , and exposed for 20 min to phorbol 12-myristate 13-acetate (PMA), 4 α -phorbol didecanoate (inactive phorbol; 4 α PDD) or dimethyl sulphoxide (DMSO), the solvent used. We found that PMA, but not 4 α PDD, stimulated the phosphorylation of two easily identifiable proteins in ^{32}P -labelled membranes (Fig. 3a). These seem to correspond to band 4.1 (M_r 80K), as reported previously in rabbit erythrocytes¹⁴, and a minor ^{32}P -phosphoprotein of M_r 125K. ^{32}P -labelled membrane protein was solubilized and immunoprecipitated with the monoclonal antibody. Specific immunoprecipitation of ^{32}P -labelled transporter indicated that the transporter was phosphorylated in intact cells in the basal state and that the phosphorylation increased substantially in response to PMA (Fig. 3b).

These data suggest that phorbol ester-stimulated glucose uptake may be, in part, a consequence of protein kinase C-

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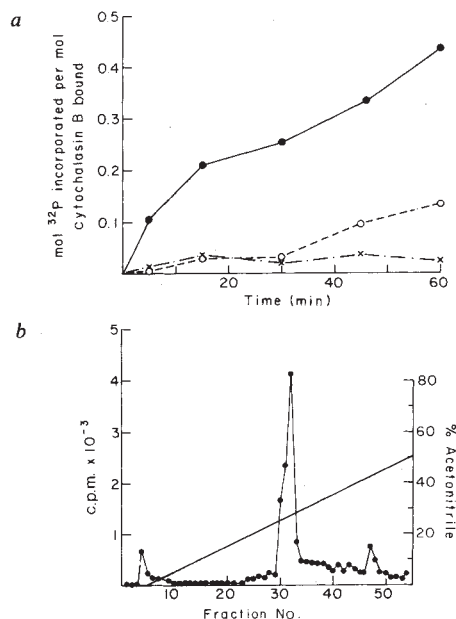


Fig. 2 Time course and stoichiometry of glucose transporter phosphorylation (*a*) and HPLC separation of tryptic ^{32}P -phosphopeptides (*b*). In *a*, the glucose transporter preparation was phosphorylated with protein kinase C in the presence (●) and absence (○) of added CaCl_2 and in the absence of added kinase (×), as in Fig. 1, and ^{32}P incorporation quantitated by trichloroacetic acid (TCA) precipitation in the presence of carrier bovine serum albumin. Control values for kinase autophosphorylation have been subtracted from total ^{32}P incorporation. Results are expressed as mol ^{32}P incorporated per mol cytochalasin B binding (taken as 1 binding site per mol functional transporter). Cytochalasin B is a high-affinity ligand for the transporter. Its binding provides a measure of the amount of functional transporter in a preparation, which is typically ~60% of the protein weight¹¹. In *b*, phosphorylated transporter was precipitated with TCA, washed and digested with 20% trypsin (w/w) at 37°C for 18 h. The solubilized ^{32}P -peptides were separated by HPLC with C_{18} reverse-phase chromatography using an ascending gradient of acetonitrile in $\text{H}_2\text{O}/0.1\%$ trifluoroacetic acid. When the same procedure was applied to a control reaction mixture containing only protein kinase C, no significant peak of ^{32}P -peptide was found.

mediated phosphorylation of the glucose transporter. We are now investigating whether the phosphorylation of the erythrocyte transporter *in vivo* in response to PMA alters its activity and whether the stimulation of glucose uptake by PMA in other cell types is correlated with phosphorylation of the transporter *in vivo*. In addition to the possibility that such phosphorylation may directly affect the kinetic parameters of the transporter, in some cell types it may also lead to the redistribution of the transporter between the plasma membrane and intracellular membranes, such that a larger fraction of the transporter lies within the plasma membrane¹⁵.

Evidence has been accumulating that epidermal growth factor and platelet-derived growth factor may lead to the activation of protein kinase C in intact cells^{16–20}; this is often accompanied by an increase in phosphatidylinositol turnover, with the generation of diacylglycerol, an activator of the kinase²¹. Epidermal growth factor has also been shown to increase glucose uptake²², while phorbol esters mimic the insulin-stimulated phosphorylation of the insulin receptor β -subunit on serine residues in IM-9 lymphocytes, suggesting that the kinase may be activated

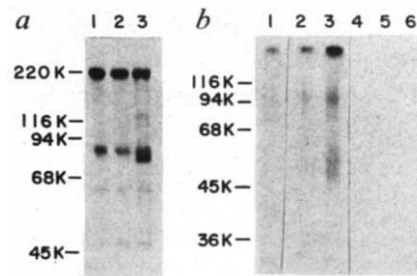


Fig. 3 Phorbol ester-stimulated protein phosphorylation of human erythrocyte membrane proteins (*a*) and glucose transporter, as determined by immunoprecipitation (*b*). In *a*, human erythrocytes were labelled with $^{32}\text{P}_i$, and membranes prepared by the method of Patel and Fairbanks²⁵. Labelled cells were exposed for 20 min to phorbol 4 α -didecanoate (1 μM , lane 1), DMSO (0.1%, lane 2) and phorbol 12-myristate 13-acetate (lane 3), before cell lysis. Membrane protein (20 μg) from each sample was separated on SDS gels before autoradiography. In *b*, membrane protein from the same samples was solubilized with 2% octyl glucoside, centrifuged to remove insoluble cytoskeletal aggregates and the soluble ^{32}P -proteins were immunoprecipitated with anti-transporter monoclonal antibody (lanes 1–3) or control monoclonal antibody (lanes 4–6). Lanes 1, 4, 4 α PDD; lanes 2, 5, DMSO; lanes 3, 6, PMA. Preliminary experiments had established the presence of protein kinase C in human erythrocytes; although not detectable as a Ca^{2+} - and PS-stimulated kinase in red blood cell lysates, it was readily recognized following DEAE-cellulose separation of lysate proteins (not shown).

by insulin²³. The present study suggests that the diverse effects of several hormones and phorbol esters on glucose transport may in part be linked through a common mechanism, phosphorylation of the glucose transporter by protein kinase C.

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Unfashionable continua

Michael Berry

An Idiot's Fugitive Essays on Science: Methods, Criticism, Training, Circumstances.
By C. Truesdell.
Springer-Verlag: 1985. Pp.654. DM 158, \$45.20.

TO DESCRIBE Clifford Truesdell as a theoretician specializing in the mechanics of continuous distributions of matter would, although strictly correct, be misleading. It would fail to convey what this collection of reviews, prefaces and lectures abundantly does convey, the broad scale of Truesdell's researches in mechanics and its history and his concern for and lack of sympathy with the way science is practised today, all so intensely felt and expressed with such mastery of language that my attention was gripped and held.

It is important to study continuum mechanics because

Matter is commonly found in bodies consisting of materials. Analytical mechanics turned its back on this fact, creating the centrally useful but abstract concepts of the mass-point and the rigid body... "modern" physics likewise turns its back, since it concerns solely the small particles of matter, declining to face the problem of how a specimen made up of such particles will behave in the typical circumstances in which we meet it. Materials, nonetheless, continue to furnish the masses of matter we see and use from day to day: air, water, earth, flesh, wood, stone, steel, concrete, glass, rubber,... All are *deformable*. A theory aiming to describe their mechanical behavior must take heed of their deformability and represent the general principles it obeys.

That theory is continuum mechanics. In renovating its foundations during recent decades, Truesdell and his colleagues have reversed the customary approach:

From the time of NEWTON until recently, many natural scientists considered the mass-point the fundamental quantity of nature, or at least of mechanics. They believed that matter was composed of many small particles obeying the laws of classical mechanics, and that, consequently, the behavior of gross matter could be predicted, in principle, to any desired accuracy, from a knowledge of the intermolecular forces. Thus continuum mechanics appears as an approximate or at best secondary theory within classical mechanics. While this tradition clings on in physics teaching today, it defies reality... The smallest units of matter are no longer believed to obey Newtonian mechanics... In fact it is almost the rule that *Newtonian mechanics, while not appropriate to the corpuscles making up a body, agrees with experience when applied to the body as a whole...* Only paedagogical custom has hindered the realization that *as a physical theory, continuum mechanics is better than mass-point mechanics.*

This has been an unfashionable view:

... "physics", by definition, is become exclusively the study of the structure of matter, while anyone who considers physical phenomena on a supermolecular scale is kicked aside as not being a "real" physicist. Often "real" physicists

let it be known that all gross phenomena easily *could* be described and predicted perfectly well by structural theories; that, aside from the lack of "fundamental" (i.e. structural) interest in all things concerning ordinary materials such as water, air, and wood, the blocks to a truly "physical" (i.e. structural) treatment are "only mathematical".

It is curious that these same persons are often sympathetic to non-structural theories on the borders of physics. They do not suggest that a mathematical physiologist investigating models for behavior would do better to try to integrate the equations of motion for the elementary particles making up a rat.

That was written in 1962. Although the point is still worth making, its thrust is blunted by the increasing readiness of physicists to apply theories where they are appropriate, with less fundamentalist prejudice against the macroscopic. One of several reasons for this is the renewed appreciation and extensive development of Poincaré's discovery of great complexity (including chaotic behaviour) in the solutions of the equations of mechanics, even when, as with mass-points, the equations themselves are simple. I would have liked to learn Truesdell's opinion of this new branch of mechanics, but was disappointed to find no mention of it.

Penetrating reviews of books about the Bernoullis, Euler and Newton illustrate Truesdell's insistence that criticisms should be based on detailed study of original sources rather than the writings of commentators. It is a pity that he fails to live up to these standards when attacking "applied catastrophe theory" as a misuse

of mathematical modelling; apart from a brief quotation (without reference) from Zeeman, his remarks are all based on articles by other critics of the theory, and he fails to mention those cases (even in continuum mechanics) where the mathematics has found valid application.

More generally, Truesdell laments the decline of scholarship:

The world of "learning" has become a federation of hives of frightened bees, who so as to maintain their little waxen prisms sting all strangers, be they lions or mice or only bees from the next-door office.

He detests the way science has become a large-scale organized enterprise, that is:

... science for, by, and of the demos, in a word, *plebiscience*. Plebiscience demands what POST has called "inevitable research: ... research which is bound to yield *some* results"...Plebiscience is *big science*. Small science was done by a few great men. Big science calls for many little men.

Worse might be to come:

Plebiscience is an intermediate stage. The next and last is *prolescience*. In it not only is all research inevitable research, but also only outcomes previously known and accepted are allowed. The function of prolescience will be to confirm and comfort the proletariat in all that it will by then have been ordered to believe. Of course that will be mainly social science.

But after pages of splendid raging, with sour prejudice alternating with flashing insight, he is not without hope:

Even now, I think, there are men who live for science, men who strive to bake not witches' mess but giants' bread. What they deliver may be as tedious to the plebs as a lute, but let us not forget that while a cure for cancer may raise the mean age of the population to ninety, a good lute may last to make beautiful music under the hands of men and women yet unborn.

Amen. □

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IMAGE
UNAVAILABLE
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Celebration of mass on the back of a whale, from an engraving dated 1621. The picture is reproduced from a reprint of the original, 1925 edition of Whale Ships and Whaling: A Pictorial History, by George Francis Dow. Publisher is Dover, New York, price is \$8.95.

Fifty years of the special senses

F. W. Campbell

Foundations of Sensory Science.

Edited by W. W. Dawson and J. M. Enoch.

Springer-Verlag: 1984. Pp. 577. DM 288, \$82.30.

THIS is a collection of essays written by scientists who have accumulated age and wisdom and achieved success in their respective fields of neurosensory research. The editors have encouraged them to clothe their accounts with personal details of how, and why, they chose a particular research direction. The result is a unique and fascinating volume, which it is unwise to start reading too late at night.

Today custom dictates that scientists report their findings in a cold impersonal style. Worse, most of us know that we often put our last experiment at the beginning of our papers, and the false-starts at the end, thereby giving a spurious impression of undue intelligence and rationality. As Edwin Land (of the Polaroid Corporation) once said, "An original idea only takes a few seconds cessation of stupidity". Many such seconds are brought to life in the book, in a relaxed manner which is much more illuminating than the formal prose of the scientific paper.

The 15 contributors are of international repute and they cover the entire range of special sense neurophysiology, including the relevant psychophysics.* Each describes their chosen problem as it was when they started as a young worker, and then unravels each twist of serendipity that put them back onto the successful track. The excitement and pleasure of the hunt are captured vividly, as are the dull and dispiriting periods which most researchers know all too well.

The young, computer-orientated scientist of today may be surprised to learn that so many of the experiments described

were done with simple, home-made apparatus; indeed, the most useful hobby seems to have been the building of short-wave radio and radar devices. However, it would be a mistake to conclude that research was straightforward in those times and, furthermore, that all the "easy" experiments have now been done. The picture that emerges is that the secret of research is to ask the right question at the right time and never to be discouraged by failure.

The authors are drawn from many different countries, and for almost all of them the Second World War had a dramatic impact on their workplace, personal life and future. Many of them were deflected into applied research, which they appeared to be equally good at, and in many cases their eventual field of study seems to have been determined simply by chance.

This elegant book provides a snapshot (with an exposure time of 50 years) of the foundations of the techniques which have enabled us to begin to probe the great,

unsolved mystery of how animals sense their ambient environment. It is very well illustrated and included are most of the classical experiments up to the present time. The names index contains some 1,300 entries, and a newcomer to work on any of the special-senses would do well to begin here where they will be directed to the relevant seminal research. Moreover, the book brings together the sensory physiology of a very wide variety of species. One of the best starting points is Fig. 1 on page 382, where an illustration of the bombykol receptor cell of the moth will immediately lead you into the pheromone quest.

The editors, W. W. Dawson and J. M. Enoch, must have had a difficult time blending together the writings of such a variety of personalities. This they have done with an affection for experimental neurosensory science which runs through every page. □

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More of the unseen Universe

Reuven Ramaty

Gamma Ray Astronomy.

By Rodney Hillier.

Oxford University Press: 1984. Pp. 202. £19, \$29.95.

IN THIS rather brief volume, Dr Hillier has attempted to present the theoretical concepts, the principal observational results and the detection techniques of gamma-ray astronomy. After an introductory chapter and an account of astrophysical gamma-ray production, five chapters are devoted to detection techniques and four to observations. Thus most of the important aspects of gamma-ray astronomy are covered, but to me the book seems to lack the depth that would be expected from a modern work on the subject. Moreover many of the topics covered have been previously treated in Stecker's *Cosmic Gamma Rays*, published in 1971, and in Chupp's *Gamma Ray Astronomy* of 1976.

The book is not entirely up to date and contains some errors. The Crab nebula is powered by the Crab pulsar and not by ^{249}Cf (p.11). The energies of the line photons resulting from positron annihilation are equal to the electron rest mass energy and not to half of this energy (p.12). And the lifetime of the 4.43 MeV level in ^{12}C is less than 5×10^{-14} s, and not $\sim 10^{-12}$ s (p.6). This difference has important astrophysical consequences regarding the width of the line, as was pointed out by Lingenfelter and Ramaty in connection with gamma-ray line emission from inter-

stellar grains (*Astrophys. J.* **211**, L19; 1977).

There are also some omissions and some topics are covered rather sketchily, for example optical flashes from gamma-ray bursters. Even though optical observations technically do not belong to the realm of gamma-ray astronomy, Schaefer's discovery (*Nature* **294**, 722; 1981) of a historical optical flash in the error box of a gamma-ray burster has been one of the most important observational advances in this area in the past few years. As such, it should have been included in the book. Similarly, because of their close relationship to gamma-ray observations, neutron observations from solar flares (Chupp *et al.* *Astrophys. J.* **263**, L95; 1982) should also have been mentioned. Another topic not fully covered is the time variability of the 0.511 MeV annihilation line from the direction of the galactic centre. This variability, first reported by Riegler *et al.* (*Astrophys. J.* **248**, L13; 1981) effectively excludes multiple or extended positron sources, for example pulsars, supernovae, primordial black holes or cosmic rays. The reference to the work of Paciesas *et al.* (p.161) relates only to the variability of the continuum emission from the direction of the galactic centre, but not to that of the annihilation line.

Overall, this is a book to which students wishing to become acquainted with the subject can be directed (though some mathematical ability is needed to get the best out of it). On the other hand, because of the failings outlined above, I cannot recommend Hillier's *Gamma Ray Astronomy* to advanced graduate students or active researchers. □

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* "Comparative Physiology of Invertebrates: Hearing and Vision", H. Autrum; "The Development of Auditory Neurophysiology", H. Davis; "Microstructure of the Inner Ear", H. Engström; "Biophysics of the Mammalian Ear", J. J. Zwislocki; "Neurophysiology of the Retina", T. Tomita; "Recollections of Early Laboratory Experiments on Vision", L. R. Riggs; "The Perception of Light and Colour", W. D. Wright; "Binocular Vision" and "Optics and Vision", G. A. Fry; "A Personal Vision", R. Granit; "Taste Electrophysiology, Sensory Coding, and Behavior", C. Pfaffmann; "A Personalized History of Taste Biophysics", L. M. Beidler; "Insect Olfaction — Our Research Endeavour", D. Schneider; "Cutaneous Temperature Sensitivity", D. R. Kenshalo; "The Sensory Detection of Vibrations", W. D. Keidel; "Fifty Years of Vestibular Science", O. Lowenstein.

Doing the rounds of the Solar System

Carl D. Murray

Rings: Discoveries from Galileo to Voyager.

By James Elliot and Richard Kerr.

MIT Press: 1985. Pp. 209.

\$17.50, £19.50.

OBSERVATIONS of the occultation of star SOA 158687 by the planet Uranus on 10 March, 1977, were intended to provide information on the shape and atmosphere of Uranus. An unexpected bonus for Jim Elliot and his team in the Kuiper Airborne Observatory was the first discovery of a ring system since the time of Galileo. After more occultations, the spectacular successes of the Pioneer and Voyager spacecraft and the increased use of charge coupled devices (CCDs), astronomers now have the luxury of studying planetary rings on a comparative basis.

Even to document the recent discoveries and theories is a difficult task after such an influx of ground-based and spacecraft results. But the authors of this book do not merely present the observations together with the latest well-polished theories, they also treat the reader to a revealing and honest portrayal of scientific discovery, complete with rejected grant applications, rivalry and occasional good luck.

In particular, the account of the spacecraft flybys captures the unique atmosphere of "instant science", when making discoveries "is just a matter of who's around". Explaining them can take a little longer. Theorists have had to turn their attention to such phenomena as narrow rings, eccentric rings, multiple rings and "spokes". The book relates how, during the Voyager 1 encounter with Saturn, some of the best theorists were unable to explain the "braided" appearance of Saturn's F-ring. Although there were doubts that gravitational forces alone could account for such a feature, one of the faithful remarked, "Don't worry about Newton's equations, most people don't realize how many solutions they have". Current theories of the F-ring have shown that his faith in gravity was not misplaced.

Accounting for some features can lead to more fundamental problems. Studies of satellite-ring interactions and the identification of spiral density waves have provided an explanation for most of the structure in Saturn's A-ring. However, the same studies reveal the possibility that unless some locking mechanism can be found we may have to come to terms with the fact that the rings are relatively short-lived phenomena.

The combination of first-hand experience and good journalism has resulted in

an immensely readable account of how ring studies have evolved over the past three centuries. Glimpses of the personalities involved and clear explanations of the various physical processes and ring theories help to convey the excitement of science. This golden age of discovery is not over. In January 1986 we can expect images of the Uranian rings from Voyager 2. Before the end of the decade we will have further information on the peculiar

rings or arcs of material around Neptune and a look at Jupiter's ring from the Galileo spacecraft. There will be more discoveries, more puzzled astronomers, more theories and, we must hope, an equally fascinating sequel to this splendid book. □

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Levels of dispute

Paul Hoyningen-Huene

Science and Values: The Aims of Science and Their Role in Scientific Debate.

By Larry Laudan.

University of California Press: 1984.

Pp. 149. \$15, £14.25.

SCIENCE, viewed as a social enterprise, has two complementary features: the formation of agreement from disagreement and the formation of disagreement from agreement. The task of explaining these features was made prominent more than 20 years ago by Thomas S. Kuhn in his *Structure of Scientific Revolutions*. Laudan takes up the subject once more, thus putting himself in competition with Kuhn and many other writers. Before addressing the resulting quarrels, we should look at the model that Laudan proposes.

Laudan distinguishes three levels of scientific commitment on which agreement or disagreement occurs: a "factual level", concerning claims about the world; a "methodological level", concerning claims about the correct way of doing science; and an "axiological level", concerning the basic cognitive aims of science (that is, those properties of theories that are constitutive of *good* theories). Traditionally, the interaction between these levels is seen as follows: disagreement on the factual level can be rationally resolved by recourse to the methodological level, and disagreement on the methodological level by recourse to the axiological level. The second of these steps is possible since methodological rules are instruments for realizing cognitive goals. The obvious difficulty which this hierarchical dependence of levels leaves unresolved is that disagreement on the axiological level cannot be eliminated.

Laudan adds more interactions to the traditional hierarchical dependence of the levels, resulting in what he calls the "reticulated model". In this model, the methodological level is also influenced by the factual level. Scientific methods can and must, he says, be judged also with respect to the factual level: what is already known about a subject matter obviously influences the choice of subsequent scientific methods. Second, the axiological level is

connected to the other levels by feedback loops that sometimes allow for rational resolution of axiological disagreement. Explicit cognitive values — value statements — may, according to Laudan, rationally be criticized for being "utopian", that is for being incapable of application, or for not being consonant with shared exemplary scientific work. (At this point, however, Laudan appears to confound explicit value *declarations* with values displayed in actual scientific *practice*.) The interdependence of the different levels in the reticulated model permits rational transitions between many of the eight combinatorially possible states of three-level agreement-disagreement.

Laudan's development of the model is accompanied by attacks on other writers in the field. Correspondingly, he declares his book to be a contribution to the "decanonization of a discipline's patron saints" (xiii), and his principal target throughout is T.S. Kuhn. But the creature decanonized by Laudan is largely a product of his own imagination. For example, a central sin that Laudan identifies in Kuhn's work is what he calls "the covariance fallacy", the assertion of a rigid bond between changes on the factual and on the axiological level (43ff.). But Kuhn, in an article that Laudan repeatedly cites, has described at length a very different sort of association, one that invokes just the sort of delayed feedback loop that Laudan takes to be his own. Elsewhere, documenting Kuhn's distrust of rationality, Laudan attributes to him the view "that it was *perfectly reasonable* for Priestley to hold onto phlogiston theory" (pp. 72–73, my italics). But what Kuhn said in the relevant passage is the reverse: "the historian can always find men — Priestley, for instance — who were *unreasonable* to resist for as long as they did" (*Structure*, p. 159, my italics). These examples stand for a host of others. Scholarly debate should not be conducted in this way. □

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●The most recent volume to appear in Pergamon's *Key Environments* series is *Antarctica*, edited by W.N. Bonner and D.W.H. Walton. Price is £14.95, \$23.95. For review of the first three volumes see *Nature* 312, 205 (1984).

Biological Sciences

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